

## ANNUAL GENERAL MEETING.

25th MARCH, 1959.

The Eighty-Fourth Annual General Meeting was held in the Society's Rooms, Science House, 157 Gloucester Street, Sydney, on Wednesday, 25th March, 1959.

Dr. S. Smith-White, President, occupied the chair.

The minutes of the Eighty-Third Annual General Meeting, 26th March, 1958, were read and confirmed.

## PRESIDENTIAL ADDRESS.

Presidency of the Linnean Society of New South Wales confers both an honour and an obligation. I should like to express my thanks to the Society for the former. I feel that there is an obligation on the President, in presenting the address, to maintain the standards of past years. I hope I will not fail the Society in this respect.

I should like to thank Dr. W. R. Browne and Dr. A. B. Walkom for their continued services as Honorary Secretary and Honorary Treasurer-Editor. They have served us for so long and have served us so well that we must be careful not to take their services for granted. I should also like to express appreciation to Miss G. L. Allpress, our Assistant Secretary, for her continued loyalty; the efficient management of the daily affairs of our Society and the maintenance of our Library reflect our indebtedness to her.

The Society's PROCEEDINGS for 1958, Volume 83, Parts 1 and 2, were published in 1958, and Part 3 in March, 1959. Volume 83 consists of 387 pages, 6 plates and 392 text-figures. A grant of £125 was made by the Commonwealth Publications Fund Committee towards the cost of publication of "Catalogue of Australian Mammals and their Recorded Internal Parasites", by Dr. M. Josephine Mackerras, and payment for a two-colour plate to illustrate a paper by E. Gauba and L. D. Pryor, entitled "Seed Coat Anatomy and Taxonomy in *Eucalyptus*. I", was made by the Department of the Interior, Canberra, A.C.T. Printing of illustrations by collotype was considered by Council and it was resolved that appropriate action be taken to let prospective authors know of the availability of collotype illustrations for their papers, under certain conditions, including one that the author bear half the cost above that of a corresponding half-tone block. A minimum number of eight plates was another proviso, as for any number less than eight the cost would be prohibitive.

During the year twelve new members were added to the list, two members died, nine members and one associate member resigned, and two were removed from the list under Rule vii. The numerical strength of the Society at 28th February, 1959, was: Ordinary Members, 223; Life Members, 32; Corresponding Members, 2; total, 259.

The resignation from Council of Mr. A. J. Bearup was regretfully accepted by Council at its February, 1959, meeting and Dr. I. V. Newman was elected in his place. Mr. Bearup resigned on account of ill health. We greatly appreciate his services to and interest in the Society and offer him our best wishes for his speedy recovery.

New holland blinds for our suite of offices and meeting room, a steel filing cabinet and twenty-five new, comfortable, modern seats have been purchased during the year.

On 19th June, 1958, the first Sir William Macleay Memorial Lecture was delivered by Professor A. A. Abbie, of Adelaide, to a large audience in the hall in Science House. The title of the lecture was "Timing in Human Evolution" and the preliminary part was devoted to interesting facts in the lives of the Macleays, and Sir William Macleay in particular (see PROCEEDINGS, 83, 1958: 197-213).

Lecturettes were given at the following meetings: April, National Parks in New South Wales, by Mr. Allen A. Strom; September, The Natural History of Australian Echinoderms, by Miss Elizabeth C. Pope; and October, Whales and Whaling, by Mr. W. H. Dawbin. No ordinary monthly meetings were held in June or August, 1958. A symposium was held in July, 1958, on "Origin and Distribution of Australian Fauna and Flora", in which a leading part was taken by Professor R. L. Crocker, Dr. J. W. Evans and Mr. Gordon Packham. We express our thanks and appreciation to all lecturers for their contributions to the programmes of the meetings. Members also showed keen interest in bringing notes and exhibits and entering into the interesting discussions which often took place.

The relatively small attendances at ordinary monthly meetings have been causing the Council some concern, and in an endeavour to meet the convenience of members the time of meeting was altered to 6 p.m., without, however, any marked improvement resulting. The matter of increasing attendances is still under consideration and suggestions by members would be welcomed.

Library accessions from scientific institutions and societies on the exchange list amounted to 1,727 compared with 1,858 in the previous year. Requests for library loans from members and institutions were as numerous as in the past years. The following donations to the library were made: (1) Sir Gavin de Beer, two books, "The Origin of Species", by Charles Darwin (a reprint of the sixth edition with a preface by Sir Gavin de Beer) (1958), and "Evolution by Natural Selection", by Charles Darwin and Alfred Russell Wallace (with a foreword by Sir Gavin de Beer) (1958); and (2) Captain R. Lloyd Jones, three books by Alvin Boyd Kuhn, Ph.D., "The Lost Light" (1940), "Who is this King of Glory?" (1944) and "Shadow of the Third Century" (1949). Council decided to purchase "Scientific Serials in Australian Libraries" and amendment sheets as published from year to year. Exchange relations were entered into with a number of institutions. The PROCEEDINGS were offered to Museo Civico di Storia Naturale di Venezia, Venice, Italy; Universidad de Buenos Aires, Facultad de Ciencias Exactas y Naturales de Buenos Aires, Argentina, and North Queensland Naturalists' Club, Cairns, North Queensland (in addition to Abstract of Proceedings already sent). Reprints were offered in exchange to: Service de Parasitologie Végétale, Institut Pasteur, Paris, France (Zoological and Entomological); Institute of Entomology, Academia Sinica, Peking, China (Entomological); Facultas Rerum Naturalium Universitatis Comenianae, Bratislava, Czechoslovakia (Botanical); National Geological Library, Peking, China (Geological); Université de Dakar, Faculté des Sciences, Dakar, French West Africa (Marine Zoology); Museo de Historia Natural "Javía Prado", Universidad Nacional Mayor de San Marcos, Lima, Peru (Geological); and Mammals Research Institute, Polish Academy of Sciences, Białowieża, Poland (Zoological and Entomological). A resumption of the exchange of publications with Naturwissenschaftlicher Verein, Hamburg, Germany, took place during the year.

The total net return from the Society's one-third ownership of Science House for the year was £1,303 10s. 9d. A second Neon sign advertisement has been erected on Science House.

The Society contributed the sum of £5 5s. stg. towards the commemoration of the Bicentenary of the foundation of the Royal Botanic Gardens, Kew, England.

#### *Linnean Macleay Fellowships.*

In November, 1957, the Council reappointed Miss Nola J. Hannon and Mrs. Mary B. Williams to Fellowships in Botany for 1958.

Miss Hannon resigned her Fellowship on 11th April, 1958, to take up an appointment as a lecturer in the N.S.W. University of Technology (now University of New South Wales). She has since obtained her Doctorate of Philosophy.

During 1958 Mrs. Williams continued a study of the freshwater algal family Characeae. This work was drastically interrupted when the Department of Botany of the University of New England was destroyed by fire in February, 1958. The whole

of her personal collection of herbarium material and slides was lost. Part of the collection of Characeae of the N.S.W. National Herbarium was recovered in severely damaged condition and that of the Melbourne National Herbarium in moderately damaged condition. Fortunately, a large proportion of the data already accumulated from this material was saved. The loss of the material itself, however, has been a severe handicap to making a complete study of the group. During the remainder of the year an attempt was made to sort through the data on hand, but many gaps were found and the work has not been completed. Meanwhile, in collaboration with Mr. T. C. Chambers, certain races of *Nitella cristata* Al.Br. were grown in various environments differing in factors of light, temperature, and photoperiod. The results of this experiment will be incorporated in a joint paper to be presented later. Mrs. Williams did not seek reappointment to a Fellowship for 1959.

In November, 1958, Miss Alison McCusker, M.Sc., was appointed to a Fellowship in Botany for 1959. Miss McCusker proposes to undertake cytological investigations in the family Epacridaceae, including (1) the sexual system of *Leucopogon melaleucoides* (A. Cunn. ex DC.), (2) a comparison of pollen developments and breeding systems in populations of *Astroloma pinifolium* (Benth.) in eastern New South Wales and western Victoria, and (3) the production of two types of pollen in *Acrotriche divaricata* (R.Br.). We extend to Miss McCusker our best wishes for a successful year's research work.

#### *Linnean Macleay Lectureship in Microbiology.*

Dr. Y. T. Tchan, Linnean Macleay Lecturer in Microbiology, University of Sydney, has furnished a report of his activities for the year ending 31st December, 1958, as follows: Teaching and administrative duties occupied a large proportion of the year but time was found for the following research: (1) Soil fertility test. This work has been continued. Experiments showed that the algal assay technique can be used for the estimation of nitrogen, phosphorus, sulphur and magnesium. In addition the possibility of estimating certain trace elements has been under investigation. Many difficulties still have to be overcome but it is likely that the method may prove suitable for iron, manganese and molybdenum. A paper has been accepted for publication (Plant and Soil, 1959). (2) The study of nitrogen fixation by free living micro-organisms has made some progress. A paper has been published on the subject of cytochromes of *Azotobacter* (Moss and Tchan, Proc. LINN. Soc. N.S.W., lxxxiii, 1958, Part 2, p. 161). (3) With the N-fixing algae, work is in progress to obtain a general technique for isolation of bacteria-free algal cultures using antibiotics.

#### *Obituaries.*

It is recorded with regret that the following members died during the year:

SIR DOUGLAS MAWSON, D.Sc., B.E., F.R.S., an Honorary Life Member of the Society, who had been a member since 1905, died in Adelaide, South Australia, on 14th October, 1958. He was born in 1882 at Frizinghall, near Bradford, England, came to Australia as a boy, and was educated at Fort Street School, Sydney. He graduated B.E. (1902) and B.Sc. (1905) at the University of Sydney and D.Sc. (1909) at the University of Adelaide. He contributed only two papers to the Society's PROCEEDINGS (in 1905 and 1906) but always retained a keen interest in the Society in spite of his residence in South Australia and his many sojourns in Antarctica. For fuller biographical accounts of Sir Douglas Mawson see *Aust. J. Sci.*, v. 21, No. 5, p. 134 (December, 1958) and *Nature*, v. 182, No. 4645, p. 1273 (8th November, 1958).

MR. DONALD FORD ROSS died in Sydney on 4th July, 1958. He had been a member of the Society since 1945.

DR. WALTER GEORGE WOOLNOUGH, who died on 28th September, 1958, at the age of 82, was a notable figure in the geological life of Australia. He had filled with distinction important academic positions in Adelaide, Sydney and Perth, and was the first Geological Adviser to the Commonwealth Government. He joined this Society in



1899 and contributed five papers to the PROCEEDINGS. Though he resigned in 1933, he still maintained an interest in the Society and on numerous occasions rendered valued help by translating communications in foreign languages.

PRESIDENTIAL ADDRESS.

*Pollen Development Patterns in the Epacridaceae.*

A Problem in Cytoplasm-Nucleus Interaction.

Tetrad pollen is generally characteristic of the Order Ericales. In the tribe Styphelieae of the Epacridaceae this tetrad form has been altered by the regular failure of three pollen grains in each tetrad, to produce apparently single monad grains. In its most extreme form, monad development involves cytoplasmic polarity and nuclear migration in the mother cell following meiosis. This extreme monad form probably originated roughly contemporaneously with the origin of the tribe. In some genera and species it has suffered further modification, to give variable or segregating pollen tetrads and reversion to regular pollen tetrads. These patterns of pollen development are described and illustrated. An attempt is made to offer a general hypothesis to explain the mechanism of monad development and to follow up the evolutionary consequences of this hypothesis.

(For full text see pp. 8-35.)

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The Honorary Treasurer, Dr. A. B. Walkom, presented the balance sheets for the year ended 28th February, 1959, duly signed by the Auditor, Mr. S. J. Rayment, F.C.A. (Aust.), and his motion that they be received and adopted was carried unanimously.

No nominations of other candidates having been received, the Chairman declared the following elections for the ensuing year to be duly made:

*President:* T. G. Vallance, B.Sc., Ph.D.

*Members of Council:* R. H. Anderson, B.Sc.Agr.; A. N. Colefax, B.Sc.; J. W. Evans, M.A., D.Sc., Sc.D.; Dorothy A. Thorp, B.Sc.; T. G. Vallance, B.Sc., Ph.D.; and J. M. Vincent, D.Sc.Agr., Dip.Bact.

*Auditor:* S. J. Rayment, F.C.A. (Aust.).

A cordial vote of thanks to the retiring President was carried by acclamation.





## LINNEAN SOCIETY OF NEW SOUTH WALES.

## LINNEAN MACLEAY FELLOWSHIPS ACCOUNT.

Balance Sheet at 28th February, 1959.

LIABILITIES.			ASSETS.		
Accumulated Funds—			Fixed Assets—		
Amount bequeathed by Sir William Macleay ..	£	s. d.	Commonwealth Loans, at cost ..	£	s. d.
Surplus Income Capitalized ..	35,000	0 0	Debentures:	30,449	0 0
	20,843	14 1	Metropolitan Water, Sewerage, and Drainage Board, at cost ..	16,956	14 9
			Rural Bank of N.S.W., at cost ..	2,172	15 0
			Loan on Mortgage ..	6,035	0 0
			Current Assets—	55,613	9 9
			Commercial Banking Company of Sydney Ltd. ..	230	4 4
				£55,843	14 1

## INCOME ACCOUNT. Year Ended 28th February, 1959.

	£	s. d.		£	s. d.
To Salaries of Linnean Macleay Fellows ..	843	8 9	By Interest ..	2,099	10 8
" Research Expenses ..	45	0 0			
" Balance, being Surplus Income transferred to General Account ..	499	10 8			
" Capital Account ..	711	11 3			
	£2,099	10 8		£2,099	10 8

## AUDITOR'S REPORT TO MEMBERS.

I have examined the books of account and vouchers of the Linnean Society of New South Wales for the year ended 28th February, 1959, and certify that the above Balance Sheet and accompanying Income Account are correct and in accordance therewith, and in my opinion present the true state of the Society's affairs at 28th February, 1959, as shown by the books. Certificates of the investments have been inspected.

Sydney, 11th March, 1959.

S. J. RAYMENT, Chartered Accountant (Aust.),  
Auditor.

4th March, 1959.

A. B. WALKOM,

Hon. Treasurer.

## LINNEAN SOCIETY OF NEW SOUTH WALES.

## BACTERIOLOGY ACCOUNT.

Balance Sheet at 28th February, 1959.

LIABILITIES.			ASSETS.		
Accumulated Funds—	£	s. d.	£	s. d.	£
Amount bequeathed by Sir William Macleay .. .. .	12,000	0 0	Commonwealth Loans, at cost ..	15,320	0 0
Accumulated Income Capitalized ..	6,310	0 0	Debentures: Metropolitan Water, Sewerage and Drainage Board, at cost ..	800	0 0
Research Fund .. .. .	10	0 0	Loan on Mortgage .. .. .	2,200	0 0
Current Liability—	18,320	0 0	Current Assets—	18,320	0 0
Income Account at 28th February, 1959 .. .. .	86	19 7	Commercial Banking Company of Sydney, Ltd. .. .. .	86	19 7
	£18,406	19 7		£18,406	19 7

## INCOME ACCOUNT. Year Ended 28th February, 1959.

	£	s. d.		£	s. d.
To University of Sydney (towards salary of Lecturer) .. .. .	700	0 0	By Balance from 1957-58 .. .. .	127	3 0
" Insurance .. .. .	13	5	" Interest .. .. .	660	10 0
" Balance to 1959-60 .. .. .	86	19 7			
	£787	13 0		£787	13 0

## AUDITOR'S REPORT TO MEMBERS.

I have examined the books of account and vouchers of the Linnean Society of New South Wales for the year ended 28th February, 1959, and certify that the above Balance Sheet and accompanying Income Account are correct and in accordance therewith, and in my opinion present the true state of the Society's affairs at 28th February, 1959, as shown by the books. Certificates of the investments have been inspected.

Sydney, 11th March, 1959.

S. J. RAYMENT, Chartered Accountant (Aust.).

Auditor.

4th March, 1959.

A. B. WALKOM,  
Hon. Treasurer.



## PRESIDENTIAL ADDRESS.

### POLLEN DEVELOPMENT PATTERNS IN THE EPACRIDACEAE.

#### A PROBLEM IN CYTOPLASM-NUCLEUS INTERACTION.

By S. SMITH-WHITE.

(Plates i-ii; twelve text-figures.)

[Delivered 25th March, 1959.]

#### *Synopsis.*

Tetrad pollen is generally characteristic of the Order Ericales. In the tribe Styphelieae of the Epacridaceae this tetrad form has been altered by the regular failure of three pollen grains in each tetrad to produce apparently single monad grains. In its most extreme form, monad development involves cytoplasmic polarity and nuclear migration in the mother cell following meiosis.

This extreme monad form probably originated roughly contemporaneously with the origin of the tribe. In some genera and species it has suffered further modification, to give variable or segregating pollen tetrads, and reversion to regular pollen tetrads.

These patterns of pollen development are described and illustrated. An attempt is made to offer a general hypothesis to explain the mechanism of monad development and to follow up the evolutionary consequences of this hypothesis.

The interests of this Society are very diverse, and over the years presidential addresses have covered an equally diverse field. Many have presented reviews of the fields of knowledge and research in which the president of the year has had standing. It is not my intention to follow this custom. Instead, I propose to present and discuss a problem peculiar to a group of Australian plants which has interested me for many years but on which there is practically no published information.

My story concerns the characteristics of mature pollen and the patterns of pollen development found in the tribe Styphelieae of the Epacridaceae, and it involves differentiation at the intracellular level. Problems of differentiation provide one of the central themes of present-day biology, and I believe you will find my story unusual and interesting. I cannot provide the answers, but I am prepared to do some guessing. It has been said that the tortoise can make no progress until he sticks out his neck! I will become involved not only in cell polarity and cytoplasmic gradients, and the immediate reactions of nuclei to these cytoplasmic conditions, but also, in the long term, in the consequences of cytoplasmic polarity on nuclear genotype.

#### THE POLLEN OF THE STYPHELIEAE.

In the Order Ericales, and particularly in the major family of the Order, the Ericaceae, the pollen is characteristically matured in tetrads. Only in the monogeneric family Clethraceae do the individual pollen grains separate during maturation. In the Epacridaceae tetrad pollen is also produced, and this form is, to my knowledge, uniformly characteristic of the tribe Epacrideae. In the Styphelieae, however, there is an unusual diversity of pollen types.

#### *The Monad Pollen Type.*

As early as 1810 Robert Brown observed that in this tribe the pollen may be either tetrad or single. Brough (1924) described the pollen of *Styphelia longifolia* as being single. In my experience, truly single pollen grains do not occur within the

tribe. Each apparently single pollen grain is a tetrad containing one good pollen grain and three aborted and empty cells, all enclosed in a common thick exine. The terms "pseudomonad" and "monodynamosporous tetrad" have been used for the somewhat similar pollen type found in the Cyperaceae (Selling, 1947; Erdtman, 1952), but they seem unwieldy and unnecessary. The term *monad* is sufficient to distinguish this type from single pollen grains and will be used in this address.

The main features of the development of monad pollen in *Styphelia* and in *Leucopogon* have been described in the PROCEEDINGS of this Society and elsewhere (Smith-White, 1948a, 1955a, 1955b). In the pollen mother cell (PMC) immediately following meiosis, the four microspore<sup>1</sup> nuclei are uniformly spaced, either in tetrahedral or quadrant arrangement (Text-fig. 1, a). Before partition of the mother cell cytoplasm occurs, migration of the nuclei takes place; three cluster at one end of the cell and the fourth moves to, or remains in, an isolated position at the opposite end (Text-fig. 1, b). Cell walls are then laid down and the microspores are constituted. These microspores are *initially* very unequal in size (Text-fig. 1, c) and unlike in potential. The differentiation of the one functional and the three non-functional microspores occurs at a very early stage, either immediately following or perhaps during meiosis. All four microspores undergo some development, and there is some thickening of the cell walls surrounding the small ones. Very soon, however, the three minor microspores die (Text-fig. 1, d, e). As Barber has shown in *Utricularia* (1941) the opportunity for intercellular compensation is then lost and the small microspores can derive no advantage from their association with the large one to compensate them for their own deficiencies.

Subsequent growth of the tetrad and its increase in size with maturation is entirely due to the large microspore. The aborted cells become compressed and obscured (Text-fig. 1, g, h). In the functional microspore the pollen grain mitosis occurs at a rather late stage of development, and after very considerable increase in size. This PG mitosis shows an inversion of the usual polarity characteristic of the PG mitosis in many flowering plants (Sax and Husted, 1936; Brumfield, 1941; La Cour, 1949; de Almeida and Sampaya, 1950), in that the short pole of the mitotic spindle is directed radially outwards from the centre of the tetrad, and the generative nucleus, and later the generative cell, is derived from this outer pole (Text-fig. 1, f, g). Finally, the generative cell migrates into the centre of the pollen grain and its boundary becomes indistinct (Text-fig. 1, h).

This pattern of pollen development is the most frequent one in the tribe. It has been termed the *Styphelia* type, or S-type, since it was first found and is most fully developed in that genus. It is not usually associated with visible meiotic abnormalities.

Normal tetrad pollen (T-type), similar to that of the Ericaceae and the Epacrideae is also found in the Stypheliaceae, but is much less frequent. It is known to me in 11 species in the genera *Acrotriche*, *Cyathodes*, *Pentachondra*, *Trochocarpa*, *Leucopogon*, *Brachyloma* and *Lissanthe* (Table 8). Three other pollen types are also found in the tribe and involve patterns of development intermediate between the S-type and the T-type, in a sense linking them together. These will be described and considered after the implications of the S-type monad pattern of development have been discussed.

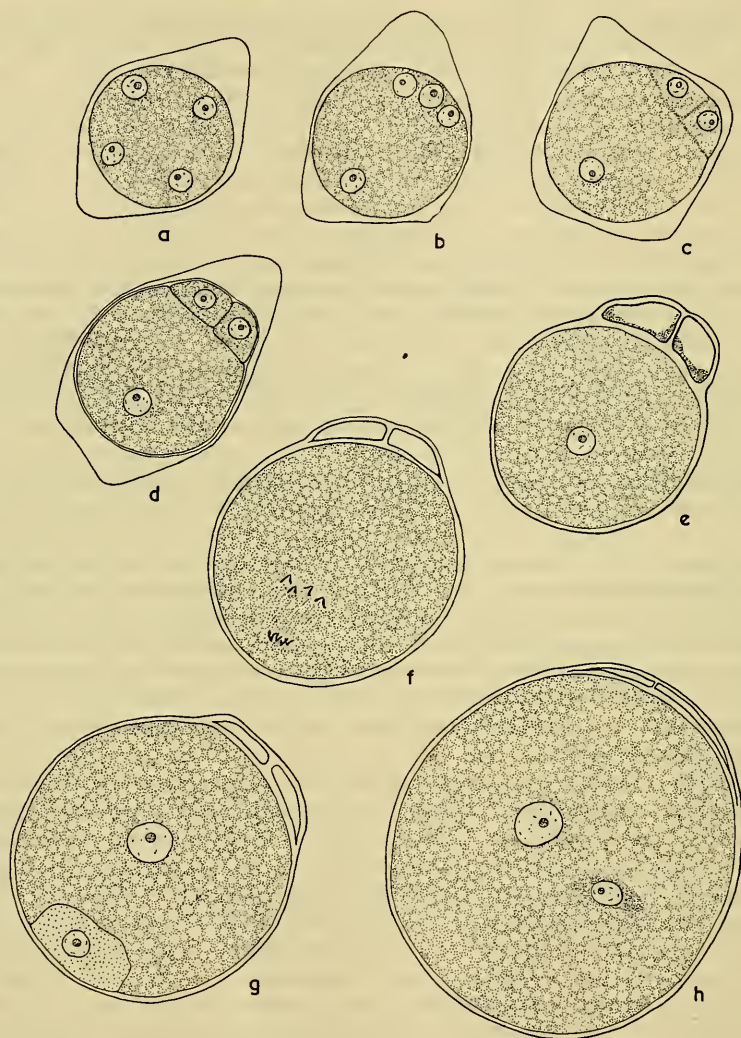
#### *The Implications of the Styphelia Pattern of Development.*

The unlike fates of the microspore nuclei in the *Styphelia* mother cell demonstrate the existence of differentiation within the cell. This differentiation may be in the cytoplasm or in the nuclei, or more probably in both.

Intracellular polarity, i.e., the development of substantive gradients between different parts of the cell cytoplasm, is not infrequent and is perhaps a necessary concomitant of all primary cellular differentiation (Mather, 1948). Such gradients

<sup>1</sup> I propose to use the term *microspore* for the four daughter cells of a PMC until they reach the stage of the first gametophytic mitosis, the pollen grain mitosis. After this mitosis they become binucleate *pollen grains*.

are evident in animal eggs, in blood precursor cells (La Cour, 1944), and in pollen grains (Geitler, 1935; Ostergren, 1947; La Cour, 1949). Similar gradients occur in embryo sacs (Darlington and La Cour, 1941; Darlington and Mather, 1949; Brock, 1954) and probably control the various patterns of embryo sac development which have been described and illustrated by Maheshwari (1950).

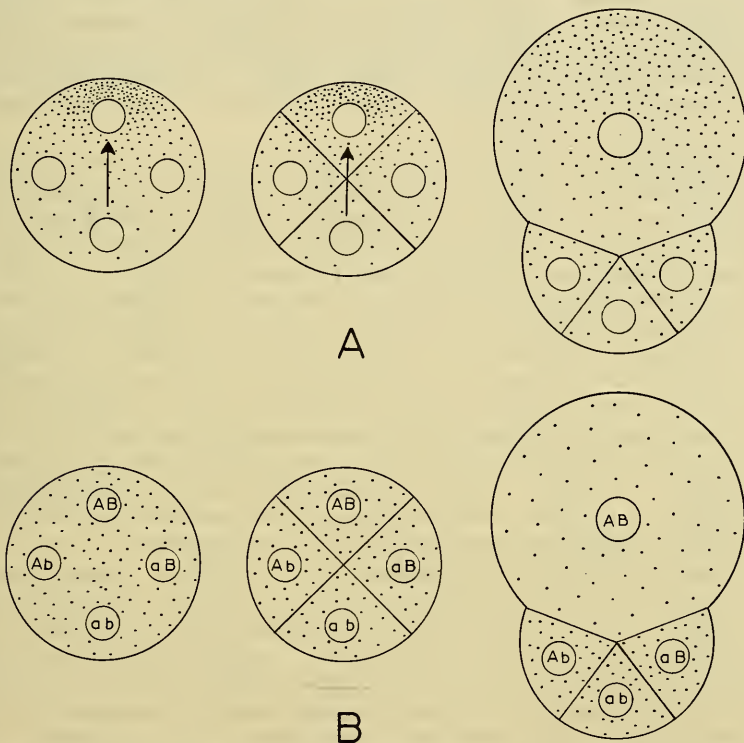


Text-fig. 1.—Pollen development in *Styphelia longifolia*.  $\times$  ca. 1150.

Very often intracellular differentiation can be related to the existence of physiological or tissue gradients in the surrounding tissues or, more generally, to a polarity of the surrounding environment: intracellular polarity is imposed from outside. Such is not always the case. La Cour (1949) and de Almeida and Sampaya (1950) have shown that the orientation of the pollen grain mitosis and the differentiation of the generative and vegetative nuclei are determined in relation to the centre of the original pollen mother cell. Each mother cell must be a centre of development of polarity. In the monad pattern of development each mother cell also seems to be a centre of origin of differentiation. There is a random orientation of the monads to the anther axis, and no general tissue gradient can be inferred. The monads are not



arranged in opposite pairs, as would happen if differentiation originated at the premeiotic mitoses. The control of monad development must be intracellular and local; in fact the usual and normal pollen grain polarity in many angiosperms and the exceptional polarity in the pollen mother cells of the *Styphelieae* responsible for monad development, are so similar in action that the former might well be the source of the latter. A slight precocity in the development of the PG polarity, bringing it forward only one cell generation, may be the fundamental change involved in the origin of *Styphelia* type pollen. Two examples of a greater precocity, causing the intrusion of polarity into the premeiotic mitosis, have been reported by La Cour (1949) and by Holden and Mota (1956).



Text-fig. 2.—Two hypotheses of monad development. A. Cytoplasmic determination. B. Nuclear (chromosomal) determination.

There seems no alternative to the view that cytoplasmic polarity is involved in the control of the monad pattern of pollen development, but it may not be sufficient. In dealing with a possible case of polarized segregation in *Bombardia*, Catchside (1944) has reasoned that it must involve both (i) a polarity in the cytoplasm or in the spindle, and (ii) some difference between the genes, which show a differential response to this polarity. His reasoning is equally valid where the units are whole chromosomes or whole nuclei. In monad development this nuclear differential could consist merely in the spatial arrangement of the four microspore nuclei or of the meiotic spindles in relation to the cytoplasmic axis. With the usual tetrahedral or quadrant arrangements one nucleus would often lie closer to the "effective" cytoplasmic pole than the other three, so that it might outgrow and suppress its sisters. This hypothesis is illustrated in Text-figure 2A.

The hypothesis needs a further brief consideration. Two features suggest that it is incomplete. It might be expected that occasionally two of the four nuclei would be approximately equally favoured in position so that dyads would be produced.

However, dyads have not been seen in any species of *Styphelia*, and in *Leucopogon juniperinus* only one dyad has been seen among over 300,000 monads. The system of control is extraordinarily efficient. La Cour (1949) has shown that in *Scilla* misorientation of the pollen grain mitotic spindle in relation to the cytoplasmic polarity may be brought about by experimental shock treatment, and that this misorientation leads to a failure of nuclear differentiation. The second feature which calls for an addition to the hypothesis is the occurrence of nuclear migration. Can a qualitative difference in nuclear response arise as a result of a quantitative difference in the concentration of some cytoplasmic activator? This problem also exists in the normal pollen grain differentiation and it emphasizes the similarity of the two systems.

Both of these features can be satisfied by a simple addition to the hypothesis. A spatial orientation of the division spindles in the cytoplasmic gradient could lead to an unequal distribution of non-chromosomal or non-genic nuclear materials and thus to a constitutional difference between the four nuclei. This constitutional difference could then condition the qualitative difference in response and the system would become self-reinforcing and reliable.

A second hypothesis is presented in Text-figure 2B merely to be eliminated. It involves chromosomal segregation and requires very precise and unlikely conditions. It requires the segregation of two loci of lethals A/a and B/b, which must be placed on the same chromosome pair at such a distance that one chiasma, and one only, is always formed between them. It thus requires chiasma localization. It also requires the maintenance of permanent hybridity by a system of complementary survival of AB pollen grains and ab embryo sacs. These conditions are unlikely. Again, it would be expected that such a system would break down in polyploids, but monad pollen is characteristic of both diploid and polyploid species, and even of the permanent triploid *Leucopogon*. The monad system breaks down only in secondary polyploids. The most serious objection to this second, segregational hypothesis, however, is that it is independent of cytoplasmic conditions and renders the assumption of cytoplasmic polarity superfluous. Thus it cannot explain nuclear migration and it isolates the pattern of monad development from the very similar patterns of differentiation in pollen grains and embryo sacs, where segregation is not possible.

At the present stage the first hypothesis must be favoured. Monad development is related to pollen grain differentiation and is determined and controlled by a cytoplasmic polarity and a consequent non-chromosomal nuclear differential, dependent upon the spatial relationship of the four microspore nuclei to the cytoplasmic polarity. However, with the intrusion of cytoplasmic polarity into the mother cell during meiosis, cytoplasmic control of segregation becomes a logical possibility, provided heterozygosity is present and provided that permanent heterozygosity can be maintained. In this connection the case of *Leucopogon juniperinus* is particularly instructive.

#### *The Special Case of Leucopogon juniperinus.*

This species is a permanent triploid numerical hybrid belonging to a section of the genus in which the haploid number is four. The pollen is similar to that of *Styphelia* and is of the extreme S-type, with marked nuclear migration occurring in the pollen mother cells. The somatic chromosome number in the species is 12, and at meiosis four bivalents are regularly formed, leaving four chromosomes as univalents. In the pollen mother cells the univalents show polarized segregation, all passing to the same pole with a frequency greatly in excess of binomial expectation (Smith-White, 1948). This polarized univalent behaviour is apparently present also in the embryo sac mother cell meiosis (Smith-White, 1955b). Permanent hybridity is maintained by complementary gametic survival, whereby the univalents are regularly excluded from the functional pollen and equally regularly are included in the functional embryo sacs. This system is illustrated diagrammatically in Text-figure 3.

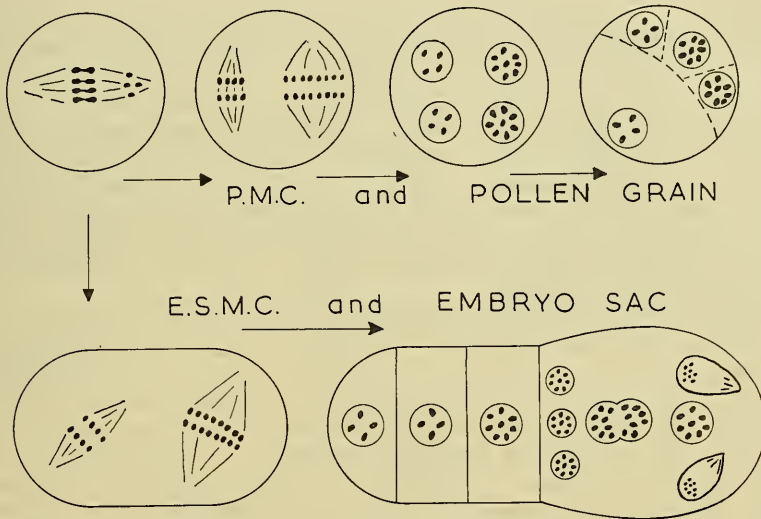
A similar controlled and polarized segregation of univalents occurs in the first meiotic divisions in embryo sac mother cells in *Rosa canina* (Tackholm, 1922; Hurst, 1931; Gustafson, 1944). In this species complex there are seven bivalents which show

normal segregation, and 21 or 28 univalents all of which pass to the micropylar pole of the embryo sac mother cell at Anaphase 1 and are thus included in the embryo sacs. The system of *Rosa canina*, which has been called semi-apomictic by Darlington (1939), differs from that of *Leucopogon juniperinus* in that in *Rosa canina* there is no control of univalent segregation in the pollen mother cells.

There can be no question or doubt that the S-type of monad pollen is antecedent to the condition of triploidy in *Leucopogon juniperinus*. The species yields evidence of fundamental importance for the analysis of the problem of monad development.

(1) It shows that the monad pattern need not be upset by triploidy. Therefore, chromosomal segregation is unlikely to be a primary cause of the differentiation.

(2) It emphasizes the presence in the pollen mother cell of a cytoplasmic gradient and it defines the time of action of this gradient, which must be developed during or before the metaphase of the first meiotic division. Polarity operates at a time when it could influence the segregation of unbalanced chromosomes.



Text-fig. 3.—The complementary gametic system in *Leucopogon juniperinus*. The four univalents are regularly included in the embryo sacs, and excluded from the pollen.

(3) It demonstrates that unbalanced chromosome univalents can and do react to the cell polarity, whereas paired chromosome bivalents, which are subject to the much stronger forces of centromere repulsion, are not free to do so.

Swanson (1958) has discussed the literature dealing with preferential chromosome segregation and a repetition is not necessary here. A short account of cases which may be relevant to our problem will suffice.

There is some evidence that preferential segregation is often a function of the centromere or of heterochromatin in the centromere region. This is true of trisomic segregation in triplo-IV *Drosophila melanogaster* (Sturtevant, 1936) and of X chromosomes in the same species (Novitski, 1951, 1954; Sandler and Braver, 1954) in *Sciara* (Metz, 1938; Crouse, 1943) and in B-chromosomes in Maize (Roman, 1947a, 1947b). In Maize heterozygous for a terminal knob on chromosome 10 (Rhoades, 1942, 1952) preferential segregation, leading to the inclusion of the knobbed chromosome 10 in the functional (chalazal) megaspore, is associated with neocentric activity of the abnormal heterochromatic knob. It is therefore significant that clonally-inherited chromosomes or chromosome segments, such as the univalents of *Rosa canina* and *Leucopogon juniperinus*, B-chromosomes, and the differential segments of sex chromosomes, are very often heterochromatic.



The controlled segregation of  $XY_1Y_2$  or of  $X_1X_2Y$  sex chromosomes, which is known in *Humulus japonicus* (Kihara and Hirayoshi, 1932), in *Rumex acetosa* (Kihara and Ono, 1925, 1928) and in several groups of insects, including some Orthoptera, Dermaptera and Heteroptera (lists and references given by Darlington, 1937, and White, 1954), is determined by the properties of associated centromeres (Darlington, 1937, p. 362) and is not relevant to our problem. The segregation of sex chromosomes in sex mechanisms of the  $X_1X_2O$  type found in spiders (White, 1954) and the  $X_1X_2X_3X_4O$  type in *Euceraphis*, where all the X chromosomes pass to the same pole, and the co-orientation of X chromosomes at M2 in *Cimex* (Darlington, 1940) cannot be explained in the same way. Swanson (1958) does not suggest an explanation for such behaviour, but a mechanism described by Catcheside (1950) in *Parthenium argetatum* could be operative. In this species, heterochromatic B-chromosomes show secondary association during meiotic prophase. Thus they tend to approach the metaphase I plate from the same direction, lie all on the same side of the A-chromosome bivalents, and pass together to the same anaphase pole.

Catcheside's mechanism implies preferential but not polarized segregation. It is not sufficient to explain univalent behaviour in *Rosa canina* embryo sac mother cells or in *Leucopogon juniperinus* pollen mother cells. It could, however, suffice to explain univalent segregation in embryo sac mother cells of the latter species, since the four univalents move together to either the micropylar or chalazal poles, apparently with equal frequency. In the embryo sac mother cells of *Rosa canina* and the pollen mother cells of *Leucopogon juniperinus* an additional mechanism is necessary to direct the univalents to a particular pole. Since in both there is other evidence of an intracellular cytoplasmic gradient, it is not unreasonable to suggest that this cytoplasmic gradient is the determining cause. The *Parthenium* behaviour then becomes superfluous, and in fact secondary association of the univalents of *Leucopogon juniperinus* during prophase has not been observed.

(4) Finally, *Leucopogon juniperinus* demonstrates that the same system of intracellular differentiation operates in both the pollen mother cells and the embryo sac mother cells in respect to univalent segregation, but that the consequences, the elimination or retention of the univalents in the pollen grains and in the embryo sacs, are different (Smith-White, 1955b). This difference must be dependent upon the different tissue environments of the anther and ovule. A surrounding tissue polarity is present in the ovule and is evidenced by the development of the micropylar megaspore rather than the chalazal one. A tissue gradient is not present in the anther.

The morphological uniformity and widespread but discontinuous distribution of this triploid species suggest that it is of very considerable age. In the course of time the univalents, subject to purely maternal inheritance, may have become vital in embryo sacs and lethal in pollen, but they cannot provide the primary cause of the monad pattern of development. The opposite contention, that the system which determines the S-pattern also influences univalent segregation, would, however, seem to be valid. The conditions which determine complementary gametic survival and which stabilize hybridity in the triploid are also present in diploid species; there is in fact pre-adaptation to permanent hybridity. Conditions which provide an opportunity for the reinforcement of a cytoplasmic control of the monad pattern of development by a chromosomal or segregational mechanism are demonstrable in the triploid and can be inferred in the diploids.

#### *Modification of the Monad Pattern of Development.*

Some species of *Leucopogon* (e.g. *L. revolutus*, Smith-White, 1955a) produce monad pollen which, when mature, is scarcely distinguishable from the S-type already described. In development, however, there is an absence of nuclear migration following meiosis, so that the four microspores are initially of equal size. Despite this apparent equality, three microspores in each tetrad regularly die at a very early stage of development. The term *modified monad* and the abbreviation S'-type will be used to designate this modification and the abbreviation M-type will be used to include both S-type and S'-type.

The S'-type of monad development is associated, so far as is known, with regular meiosis. It is still necessary to infer the existence of an intracellular cytoplasmic differentiation to account for the different fates of the four microspores and for the stability of the system, even though there is no direct evidence of cell polarity affecting nuclear movement. The distinction between the S- and S'-types is in fact quantitative rather than qualitative. In *L. ericoides* there is evidence of nuclear migration, but it is of lesser degree than that usual in *Styphelia*, and the initial size-inequality of the four microspores is also of lesser degree, although still quite distinct. In *Melichrus urceolatus* there is variation within anthers, some mother cells showing marked nuclear migration and others showing little or none.

This quantitative intergradation between the S-type and S'-type of development suggests that the control mechanism involved in monad development is itself quantitative, and it makes any classification of species into two classes, with S-type pollen and S'-type pollen respectively, such as was attempted earlier (Smith-White, 1955*a*), difficult and arbitrary.

#### *Breakdown of the Monad Pattern.*

It might be anticipated that the S'-type of pollen development would involve a lesser degree of cytoplasmic differentiation than the extreme S-type and that it might therefore be more easily subject to breakdown. This is the case. Comment has already been made upon the remarkable stability of the S-type system in *Styphelia* and in *Leucopogon juniperinus*. By comparison, *Leucopogon virgatus* ( $n = 10$ ), which has S'-type pollen, shows a relative instability, with occasional or even frequent breakdown of the monad system leading to the formation of dyads and very exceptionally of triads. In a population of this species at Rylstone, N.S.W., 50 plants have been examined. Of these, many show no breakdown or a negligible degree of breakdown with less than 0.1% of dyads. Others produced dyads with a frequency of 1-2% in every anther and two have a breakdown frequency exceeding 10%. Data from eleven selected plants of this population, presented in Table 1, illustrate the range of behaviours.

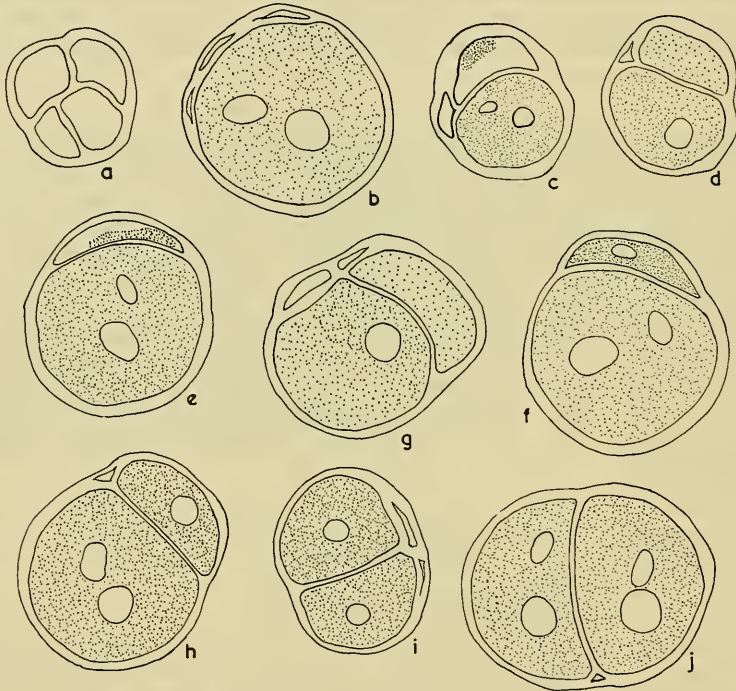
TABLE 1.  
*Breakdown of Monad Type Pollen in L. virgatus R.Br. at Rylstone, N.S.W.*  
Tetrad-type Proportions.

Plant.	Percentage Frequency of Tetrad Types.					N.	Ratio Dyads/ Monads.
	0s.	1s.	2s.	3s.	4s.		
57/4	37.85	62.15	0.00	0.00	0.00	1638	—
57/11	0.75	99.25	0.00	0.00	0.00	1196	—
57/7	2.55	97.41	0.04	0.00	0.00	2240	0.00045
57/3	3.08	96.73	0.19	0.00	0.00	3244	0.0019
58/56	2.66	97.05	0.29	0.00	0.00	13634	0.0022
57/2	6.24	93.34	0.42	0.00	0.00	2371	0.0045
57/1	4.68	94.34	0.98	0.00	0.00	4810	0.0103
58/94	0.61	96.04	3.35	0.00	0.00	9586	0.0349
58/73	39.17	57.53	3.30	0.00	0.00	3360	0.058
57/10	3.01	87.28	9.67	0.04	0.00	2625	0.111
58/8	1.37	88.46	10.06	0.11	0.00	4740	0.114

Several features in this breakdown of the monad type need to be emphasized. In some plants there is produced an appreciable frequency of nullads (i.e. completely aborted tetrads, in which the usual single pollen grain has failed to develop), but high nullad frequency does not appear to be associated with either high or low dyad frequency. Secondly, breakdown leading to dyad formation is not an all-or-nothing process. When a dyad is formed instead of the more usual monad, the two developed grains in the dyad may be equal in size and both either apparently viable or partially degenerate, or they may be slightly or grossly unequal (Text-fig. 4, *a-j*; Plate i, figs. 1-12). All degrees of breakdown, from the perfect monad to the full dyad, occur

in the same anther. The classification into monads and dyads given in Tables 1 and 2 thus involves an arbitrary decision of the degree of development which should be scored. In the data, grains showing development of a second cell equal to or greater than that illustrated in Text-figure 4, *f*, have been scored as dyads.

It will be observed that there are two different quantitative aspects of breakdown. These are (1) variation in frequency and (2) variation in degree. Breakdown does not involve a switch from one developmental pathway to another, and the control system of monad development must be itself quantitative. The behaviour supports the inference which was made earlier, i.e. the hypothesis that monad development is dependent upon intracellular cytoplasmic differentiation and polarity.



Text-fig. 4.—Monad breakdown in *Leucopogon virgatus*.  $\times$  ca. 1150. *a*. Nullad. *b*. Normal monad. *c*, *d*. Degenerate dyads. *e-h*. Degrees of monad "breakdown". *i-j*. Dyads.

In Tables 2A and 2B data are given for individual flowers and anthers, to show the extent of variation in the frequency of breakdown within plants. It is clear that significant differences occur between flowers and even between anthers of the same flower, and such differences must be environmentally determined. A comparison of Tables 1 and 2 shows that intraplant differences are of a substantially lower order than are interplant differences. It may be inferred that there are genotypic differences between plants in the population affecting the stability of the monad developmental system and that where the system is weak, buffering against environmental breakdown is marginal.

The precise nature and mode of action of environmental factors on monad breakdown remain to be determined, but they will probably be susceptible to experimental study. It is probably significant that there can be a substantial lapse of time, perhaps as much as a week, between the occurrences of meiosis in the five anthers of one flower, and that meiosis may commence at almost any time of the day or night. Thus it is possible that temperature at the time of meiosis or at some other critical stage of development could be an important factor in breakdown.



TABLE 2A.

Variation in Monad Breakdown within Plants. *L. virgatus R.Br., Rylstone, N.S.W.*  
 Variations between flowers within plants. 58/56, 58/73, 58/94 and 58/8 (triads omitted).

Flower.	Plant 58/56. Percentage Frequency of Tetrad Types.			N.	Flower.	Plant 58/94. Percentage Frequency of Tetrad Types.			N.
	0s.	1s.	2s.			0s.	1s.	2s.	
1	2.57	97.32	0.11	4508	1	0.58	96.75	2.67	2955
2	2.43	97.05	0.52	4783	2	0.56	95.87	3.57	3195
3	3.02	96.78	0.21	4343	3	0.70	95.58	3.73	3436
Total ..	2.66	97.05	0.29	13634	Total ..	0.61	96.04	3.35	9586
Contingency $\chi^2=18.40$ . $P<0.001$ .					Contingency $\chi^2=6.78$ . $P=0.15$ .				
Flower.	Plant 58/73. Percentage Frequency of Tetrad Types.			N.	Flower.	Plant 58/8. Percentage Frequency of Tetrad Types.			N.
	0s.	1s.	2s.			0s.	1s.	2s.	
1	42.20	54.49	3.31	1147	1	0.85	86.84	12.31	1649
2	42.66	54.27	3.08	1137	2	1.82	93.16	5.02	1374
3	32.25	64.22	3.53	1076	3	1.52	86.50	11.97	1712
Total ..	39.17	57.53	3.30	3360	Total ..	1.37	88.55	10.07	4735
Contingency $\chi^2=32.03$ . $P<0.001$ .					Contingency $\chi^2=59.37$ . $P<0.001$ .				

TABLE 2B.

Variation in Monad Breakdown within Plants. *L. virgatus R.Br., Rylstone, N.S.W.*  
 Variations between anthers and flowers in 58/8.

Flower.	Anther.	Percentage Frequencies of Tetrad Types.					Total.
		0s.	1s.	2s.	3s.	4s.	
1	1	0	93.30	6.42	0.28	—	358
	2	1.90	88.08	9.76	0.27	—	369
	3	0.77	82.26	16.97	0	—	389
	4	0.62	90.0	9.38	0	—	160
	5	0.80	81.96	16.71	0.53	—	377
	Totals ..	0.85	86.63	12.28	0.24	—	1653
2	1	2.83	88.68	8.49	0	—	318
	2	2.01	94.63	3.02	0.34	—	298
	3	0.60	94.89	4.50	0	—	333
	4	0.65	96.13	3.23	0	—	155
	5	2.58	92.62	4.80	0	—	271
	Totals ..	1.82	93.09	5.02	0.07	—	1375
3	1	1.28	83.89	14.83	0	—	391
	2	0.50	87.69	11.80	0	—	398
	3	0.81	84.84	14.34	0	—	244
	4	2.24	85.62	12.14	0	—	313
	5	2.73	89.89	7.38	0	—	366
	Totals ..	1.51	86.50	11.97	0	—	1712
Grand totals	.. ..	1.37	88.46	10.06	0.11	—	4740

Neglecting triads, contingency, total  $\chi^2_{23} = 137.6$ .  $P<0.001$ .

“ “ “ between flower totals  $\chi^2_4 = 59.37$ .  $P<0.001$ .

*Variable Pollen Tetrads.*

Breakdown of the monad system connects the S- and S'-types with still another, that of segregating or variable pollen tetrads. This type was first reported in *Astroloma pinifolium* (Smith-White, 1948a) and has been designated as the *Astroloma* type or A-type. Similar segregating tetrads were later reported in *Astroloma conostephioides*, *Brachyloma preissii*, and *Acrotriche fasciculiflora* (Smith-White, 1955a). Since then the type has been found in eight additional species in *Acrotriche*, *Brachyloma*, *Leucopogon* and *Lissanthe* (cf. Table 3, in which information is also given on chromosome numbers and on the pollen types of other species of the same genera).

In A-type pollen development meiosis is followed by the partition of the pollen mother cell into four apparently equal microspores. All, any or none of these may

TABLE 3.  
*Species of the Stypheliaceae showing Tetrad Segregation.*  
A-type Pollen.

Genera and Species.	Number of Plants Examined.	Number of Plants with A-type.	Chromosome Number n.	Associated Meiotic Irregularities.	Characteristics of Congeners.		
					n.	Number of Spp.	Pollen Type.
<i>Astroloma</i> .. .. .					4	14	S
<i>pinifolium</i> Benth. ..	>100	All	7	Fragmentation Inversion hybridity			
<i>conostephioides</i> F. Muell. ..	>100	All	7				
<i>Acrotriche</i> .. .. .					9	3	T
<i>cordata</i> R.Br. .. ..	3	3	Not known	Hetero- chromatin			
<i>fasciculiflora</i> Benth. ..	20	All	9				
<i>divaricata</i> R.Br. .. ..	5	1	9				
<i>depressa</i> R.Br. .. ..	2	2	Not known				
<i>Brachyloma</i> .. .. .					9	1	S, T
<i>ericoides</i> Sond. .. ..	3	3	Not known	Possible fragmentation			
<i>preissii</i> Sond. .. ..	6	6	7				
<i>scortechinii</i> F. Muell. ..	1	1	Not known				
<i>Lissanthe</i> .. .. .					7	1	T
<i>montana</i> R.Br. .. ..	10	2	14	Gynodioecism			
<i>strigosa</i> R.Br. .. ..	50	1	7				
<i>Leucopogon</i> .. .. .					4, 6 7, 10		S, S' T
<i>melaleuroides</i> A. Cunn. ..	15	15	Not known	Gynodioecism			

then abort, yielding five categories of tetrad in the mature anther—nullads, monads, dyads, triads and full tetrads (Text-fig. 5; Plate ii, figs. 1-6).

Within genera, A-type pollen is associated either with the S- and S'-types or with the full tetrad T-type. Unfortunately, chromosome numbers are not known for all species, but so far as is known the A-type is not associated with the haploid numbers of 4 or 6. In four cases it is associated with abnormal meiotic conditions.

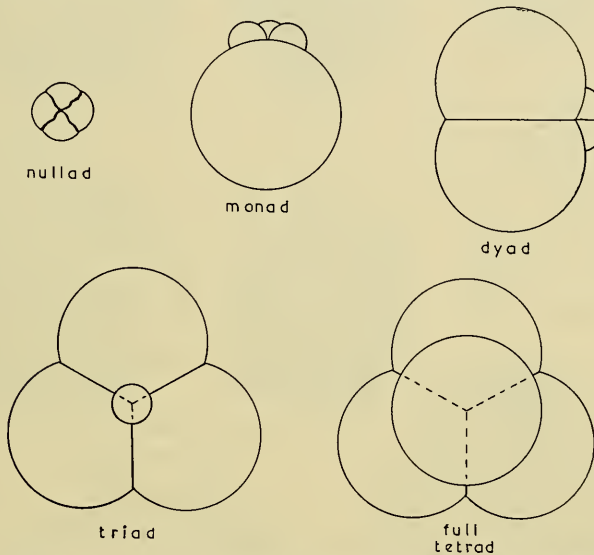
The details of pollen development subsequent to meiosis in the twelve species listed differ only in minor respects. The following description, however, applies particularly to *Astroloma pinifolium*.

Following meiosis the microspore nuclei assume a tetrahedral or a quadrant arrangement within the mother cell. There is no nuclear migration and the four microspores are initially equal in size. Inequality becomes apparent only after the cell walls separating the microspores have become appreciably thickened, but before there is any considerable increase in size, and is evident in the degeneration of the cytoplasm in some cells (Text-fig. 6, a-h). As in the development of monad pollen, aborting

microspores fail after the opportunity for intercellular compensation has been lost. It is not clear whether the deficiencies which lead to death are nuclear and chromosomal, as in *Uvularia* (Barber, *l.c.*), or whether they are cytoplasmic and related to the gradients involved in S-type monad development.

The pollen grain mitosis occurs in surviving microspores after these have undergone considerable growth and is similar to the pollen grain mitosis in *Styphelia* in respect to the reversal of generative-vegetative pole polarity as compared with *Scilla* and *Luzula* (Text-fig. 6, *g, h*).

In mature pollen, aborted cells are very small and completely devoid of cytoplasm. In some plants, however, a second kind of degeneration occurs; microspores or pollen grains die at a late stage of development, and as a consequence of this late death are little smaller than surviving pollen grains, and contain collapsed and deep-staining



Text-fig. 5.—A-type pollen. The five categories of tetrads.

cytoplasm. This type of death is sporadic in its occurrence and is apparently unrelated to the basic pollen development system of the tribe. Consequently, in scoring tetrad type frequencies late death has generally been ignored.

It could well be that full development of pollen grains at the time of anther dehiscence, as judged by their size and the condition and stainability of their cytoplasm, is not a consistent indication of their functional viability. Only one of the two or three grains in dyads and triads might in fact be functional. Observations on pollen germination in nectar (Table 4) deny this possibility. The two grains in any dyad each have a germination potential equal to that of single pollen grains in monads and are independent of each other in this respect ( $\chi^2 = 1.01$ ,  $P = 0.4$ ). It seems likely that the same independence would apply to the several pollen grains in triads and in full tetrads.

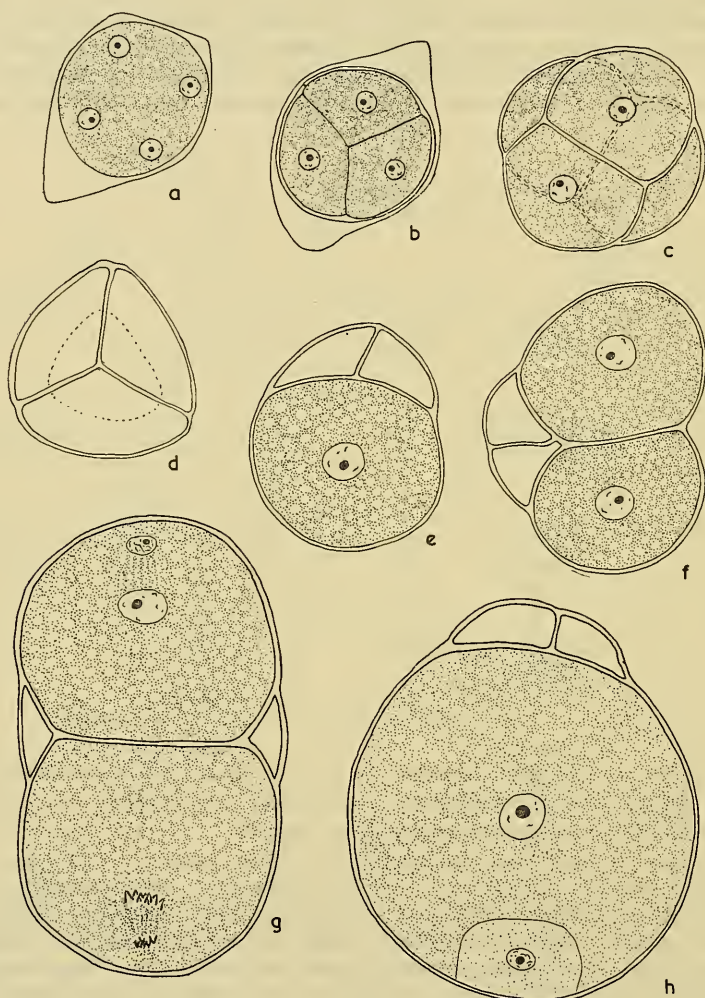
#### *Tetrad Segregations in A-Type Pollen.*

In A-type pollen the tetrahedral arrangement of the young microspores and the distortion caused by the growth of the good grains prevent any possibility of the recognition of the planes of the first and second divisions. Analysis of the segregations is thereby severely limited and can be made only on the basis of unordered tetrads. The shapes of the frequency distributions of the five categories of tetrad in the various species, and even in different plants within species, are often very different,



as will be seen from an inspection of the data in Tables 5, 6 and 7 and from the photographs in Plate ii. In respect to segregation characteristics, each species requires individual study and analysis. Such analysis will not be attempted here.

In Tables 5 and 6 a selection has been made to illustrate the diversity of segregation behaviour in different plants of the two species of *Astroloma*, and Table 7 presents data for most of the other species which were listed in Table 3. The



Text-fig. 6.—Pollen development in *Astroloma pinifolium*.  $\times$  ca. 1150.

selection of data in each table has been made to emphasize the fact that there is an almost complete range, from distributions with a modal class of monads (e.g. *A. pinifolium* 58/1005, Table 5) to others with a modal class of full tetrads (e.g. *Leucopogon melaleucoides* and *Brachyloma scortechinii*, Table 7). A-type pollen segregations thus connect the type of behaviour previously described in *Leucopogon virgatus* with the regular full tetrad T-type pollen. There is in fact a continuous series of pollen types within the Styphelieae, from the extreme S-type with nuclear migration characteristic of *Styphelia* itself, through the S'-type to S''-type with breakdown, leading to A-type variable tetrads and finally to regular full tetrads.

*Dioecism and Gynodioecism in the Styphelieae.*

Before I undertake a general discussion of the problem of pollen development which I have outlined, I want to describe another feature of the Styphelieae which may appear to be quite unrelated. This is the occurrence of dioecism and of gynodioecism in several species.

TABLE 4.  
*Pollen germination in Astroloma pinifolium Benth.*  
A. In Monads.

Germinated.	Not Germinated.	Total.	Percentage Germinated.
181	150	331	54.8

## B. In Dyads.

Both P.G.'s Germinated.	One P.G. only Germinated.	Neither P.G.'s Germinated.	Total Dyads.	Percentage P.G. Germination.
41	59	34	134	52.6
Expected on basis of independence. $(p+q)^2$ .				
37.08	66.81	33.11		
$\chi^2=1.91$ . $P=0.4$				

TABLE 5.  
*Pollen Tetrad Segregation Data. Astroloma pinifolium Benth.*  
A. East Coast Districts.

Plant.	Tetrad Type Frequencies Percentage.					N.	Percentage Pollen Fertility.
	0s.	1s.	2s.	3s.	4s.		
A. East Coast Districts.							
E51/1 ..	4.0	30.8	48.7	15.3	1.2	2336	44.7
W54/9 ..	16.2	39.7	32.8	10.5	0.8	3464	35.0
A53/1 ..	20.8	35.1	34.9	8.6	0.6	11322	33.2
O55/1 ..	20.0	39.8	30.3	9.1	0.8	1721	32.7
W54/5 ..	26.5	43.3	25.0	4.7	0.3	3603	27.2
M53/3 ..	40.0	37.3	18.7	3.7	0.3	8022	21.5
W54/6 ..	60.1	31.5	7.5	0.85	0.05	5878	12.3

## B. Grampians District, Victoria.

58/1008 ..	6.60	16.57	75.79	0.33	0.71	2408	43.0
58/1001 ..	4.31	20.82	74.16	0.05	0.66	1974	43.0
58/1005 ..	12.29	71.49	16.14	0.04	0.04	2571	26.0
58/1007 ..	30.28	59.46	10.26	0.00	0.00	2758	20.0
58/1016 ..	16.61	71.10	12.25	0.04	0.00	2776	23.9

Localities: A., Audley. E., Evans Head. O., Oatley. W., Warrah. M., Malabar.

The majority of flowering plants are hermaphrodite. Dioecism is comparatively rare, but perhaps 75% of the families, and nearly all the larger families, include some dioecic species (Yampolsky, 1922; Lewis, 1942). Whether the primitive angiosperm was unisexual or bisexual may never be known with any certainty, but in many families there can be little doubt that dioecism is a secondary and derivative condition.

Dioecism makes outcrossing obligatory. It ensures a high rate of genetic recombination and allows the maintenance of genetic heterogeneity and adaptability.

TABLE 6.  
*Tetrad Segregation Data. Astroloma conostephioides F. Muell.*

Plant.	Tetrad Type Frequencies Percentage.					N.	Percentage Pollen Fertility.
	0s.	1s.	2s.	3s.	4s.		
A. Adelaide District.							
55/107 ..	3.8	30.0	64.2	0.9	1.1	1872	41.4
55/115 ..	19.8	20.9	47.9	5.2	6.2	3185	39.3
55/116 ..	20.5	55.1	22.3	1.7	0.4	2405	26.6
55/104 ..	46.5	51.6	1.9	0.0	0.0	3200	13.9
55/101 ..	61.0	37.2	1.8	0.0	0.0	2479	10.2
B. Grampians District.							
58/214 ..	8.5	13.2	77.1	0.1	1.1	1621	43.0
58/201 ..	0.5	46.8	35.6	11.9	0.6	1281	39.0
58/401 ..	8.8	30.8	59.0	0.7	0.7	2843	38.4
58/210 ..	15.4	58.6	25.5	0.0	0.5	2182	27.9
58/212 ..	14.0	72.0	13.9	0.0	0.1	1637	25.1
58/101 ..	18.1	66.8	15.0	0.0	0.1	2102	24.2

TABLE 7.  
*Examples of Tetrad Segregation in Other Species.*

Species and Plant.	Tetrad-type Proportions Percentage.					N.	Percentage Pollen Fertility.
	0s.	1s.	2s.	3s.	4s.		
<i>Acrotriche</i>							
<i>cordata</i> R.Br.							
58/1 .. .. .	0.94	10.20	31.89	33.76	23.21	853	67.0
<i>depressa</i> R.Br.							
58/1 .. .. .	23.35	54.39	21.22	1.04	0.00	3554	25.0
<i>fasciculiflora</i> Benth.							
54/6 .. .. .	4.08	32.32	52.74	10.67	0.18	1640	42.6
54/5 .. .. .	7.84	40.67	45.79	6.06	0.00	2326	37.6
54/8 .. .. .	18.24	43.68	32.31	5.61	0.16	1891	31.4
54/12 .. .. .	90.12	7.39	2.44	0.05	0.00	4008	3.1
<i>Brachyloma</i>							
<i>ericoides</i> Sond.							
58/0 .. .. .	3.96	7.74	82.64	1.23	4.43	1060	48.6
58/1 .. .. .	1.62	6.94	90.71	0.06	0.67	1786	47.8
58/2 .. .. .	22.18	57.54	20.04	0.12	0.12	1677	24.6
<i>preissii</i> Sond.							
50/49 .. .. .	3.07	31.68	45.00	20.15	0.09	1171	45.6
52/16 .. .. .	2.29	23.46	53.66	20.52	0.07	1530	48.2
52/35 .. .. .	19.90	48.06	27.75	4.29	0.00	955	29.1
<i>scortechinii</i> F.M.							
51/1 .. .. .	0.43	1.44	5.33	21.61	71.18	694	90.4
<i>Leucopogon</i>							
<i>metaleucoides</i> A. Cunn.							
T58/12 .. ..	4.82	21.72	34.80	29.59	9.07	3342	54.1
/20 .. .. .	3.20	14.84	32.24	35.03	14.69	3942	55.7
/15 .. .. .	3.95	18.73	27.57	30.83	18.92	2024	60.9

It is the predominant feature of the sexual systems in animals, but in plants the same end is achieved more often by other means, and particularly by self-incompatibility, which are perhaps more suited to the circumstances of non-mobility and the mechanisms of pollen transference (Mather, 1940; Westergaard, 1958).

Gynodioecism is a system where a population or a species contains individuals which are hermaphrodite and others which are male sterile, i.e. functionally female.



Sporadic male sterility is known in many normally hermaphrodite plants (Lewis, 1942). Established gynodioecism as a feature of the breeding system of a species has been studied in about a dozen species of flowering plants and is known to be particularly frequent in the Labiateae. Frankel (1940) suggested that such systems may be more frequent in other families than is indicated by actual records.

Normal dioecism is maintained in natural populations by the segregation of a pair of allelic sex genes, linked blocks of genes, or sex chromosomes. Lewis (1941) has shown that gynodioecic systems cannot be easily maintained on the same basis—stability can only be achieved where the male-sterile plants are more than twice as seed-fertile as the hermaphrodite ones, and the frequency of male-sterile individuals must be less than 50% of the population. Chromosomal (or genic) gynodioecism is frequent in experimental stocks of cultivated plants, but is rare in natural populations (cf., however, Lewis and Crowe, 1956). On the other hand, cytoplasmic systems of gynodioecism are more frequent in wild species. In such systems, maternally inherited cytoplasm determines whether an individual will be hermaphrodite or male-sterile. The method permits a delicate control of outcrossing without being dependent upon an extreme advantage in seed set of male-sterile plants.

In the Ericales the normal condition is monoecism with perfect bisexual flowers. Dioecism is rare and must be derived. However, Bentham (1869) observed that some species of the Stypheleae show "partial dioecism". Apart from the normal condition of hermaphroditism, three rather different modifications of the sexual system occur within the tribe. These are: (i) complete dioecism, (ii) gynodioecism comprising hermaphrodite and male sterile plants, and (iii) androgynodioecism, where hermaphrodites, male-sterile (female) plants, female-sterile (male) plants and male-sterile/female-sterile (neuter) plants occur in the population.

In *Monotoca elliptica* R.Br. at Woy Woy, N.S.W. (Smith-White, 1955c) male-fertile plants are completely seed-sterile and dioecism is complete in the functional sense although poorly developed pistils are present in the flowers of male plants and antherless staminalodes are present in the females. Bentham (*l.c.*) has stated that in coastal populations of this species dioecism is incomplete, but in the Woy Woy population male-fertile plants are completely seed-sterile. The frequencies of male and female plants in the population are equal—one count gave 138 male and 146 female plants—and a simple sex gene or sex chromosome segregation is indicated.

A similar functionally dioecious system occurs in *Monotoca scoparia* R.Br. at La Perouse, and possibly also in *Leucopogon hookeri* Sond. at Kosciusko. Godley (1957) has reported dioecism in *Cyathodes* spp. and in *Leucopogon fasciculatus* in New Zealand. *Cyathodes divaricata* Hook. and *C. parviflora* R.Br. in Tasmania also include male sterile plants (Smith-White, 1955c) but it is not known whether dioecism is complete in these two species. A system of gynodioecism including female and hermaphrodite plants probably occurs in *Leucopogon melaleucoides* A. Cunn. at Torrington, N.S.W. (McCusker, unpublished data). In *Leucopogon hookeri* Sond. at Barrington, however, male, female and hermaphrodite plants exist.

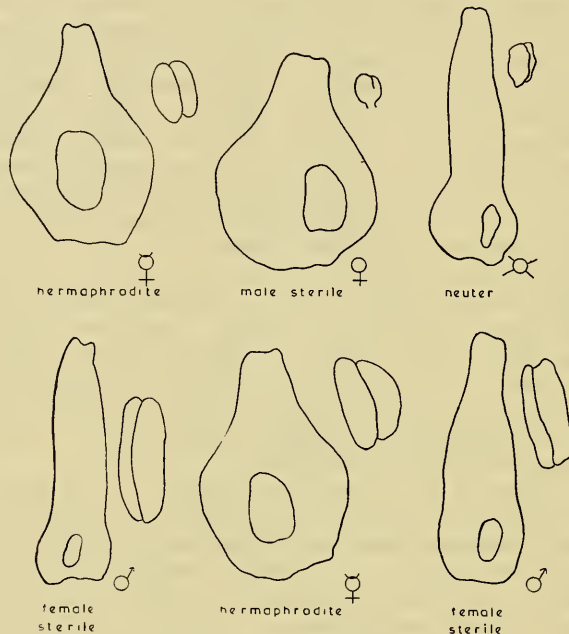
The sexual system in *Lissanthe montana* R.Br. at Kosciusko is still more complex, the population including hermaphrodites, males, females and neuters (Text-fig. 7). There is, however, a complete intergradation of sex types, since male plants show variation from "strong" to "weak" in respect to the size and pollen content of anthers, and female plants vary in pistil development and fertility. Hermaphrodite plants usually have less well-developed pistils than females and smaller anthers than males.

In field counts of the frequencies of male-fertile and male-sterile plants the following results were obtained.

	Male-fertile. ♀ + ♂	Male-sterile. ♀ + ♂
At Kosciusko Summit .. .. .	37	63
Roadside, 4½ miles below summit .. .. .	58	42
Roadside, 6 miles below summit .. .. .	48	32
Charlotte's Pass .. .. .	56	44
Spencer's Creek .. .. .	58	42

With the exception of the Summit counts, the frequencies are consistent and indicate a preponderance of male and hermaphrodite plants.

Sexual differentiation in *Monotoca scoparia* connects the complete dioecism of *M. elliptica* with the sex-strength system of *Lissanthe montana*, since male plants in some populations of *M. scoparia* show a limited seed set. There can be little doubt that the systems of sexual polymorphism found in the several species of the Styphelieae are related.



Text-fig. 7.—Representative pistils, ovules, and anthers from male, female, hermaphrodite and neuter plants of *Lissanthe montana*.  $\times 20$ . The two female-sterile plants represented differ slightly but consistently in the size of their aborted ovules.

The occurrence of complete dioecism in some species or in some populations, and of male and neuter plants in others, precludes the operation of a cytoplasmic system of sex determination. A cytoplasmic system of gynodioecism does not and cannot lead to complete dioecism since with the latter the cytoplasm of the whole species must be of the maternal, female type. A complex genetic segregational system of determination must be present in *Lissanthe montana*. Such a system may have become progressively simplified and stabilized to give complete dioecism in *Monotoca*.

Crane and Lawrence (1931) have described an experimentally produced sexual system in *Rubus idaeus* which depends on segregation at two independent gene loci. Males are ffMm or ffMM, and females are Ffmm or FFmm. Crosses between females Ffmm and males ffMm yield both hermaphrodite FfMm and neuter ffmm individuals. A similar system is known to occur in *Vitis* species (Negrul, 1936; Oberle, 1938). Another two-factor model is suggested for the Labiate *Origanum vulgare* by Lewis and Crowe (1956). These cases provide models for the interpretation of dioecism and gynodioecism in the Styphelieae, with the proviso that the actual system must be a good deal more complicated in *Lissanthe*.

#### GENERAL DISCUSSION.

##### 1. The Evolutionary Relationships of the Pollen Types.

The maturation of pollen in tetrads is not uncommon in the Angiosperms. Sometimes it may be characteristic only of individual species or genera, as in *Leschenaultia* (Goodeniaceae, Martin and Peacock, 1959, in MSS.) and in *Acacia* (Mimosaceae,

Newman, 1934), or it may be characteristic of whole families such as the Winteraceae, the Droseraceae, and the Juncaceae. A survey of its occurrence (Wodehouse, 1936; Erdtman, 1952) establishes the fact that it must have had many independent origins. Indeed the establishment of the tetrad form from the more usual single condition requires only the persistent cohesion of the post-meiotic microspores, and in genera which normally produce single pollen grains unusual conditions may lead to this cohesion. Thus La Cour (1949) has shown that heat shock may have such an effect in *Scilla*. Levan (1942) has described a gene in *Petunia* which conditions the maturation of tetrad pollen.

Since tetrad pollen is generally characteristic of the Order Ericales, excepting only the monogeneric Clethraceae, it is a possible but by no means necessary assumption that it had a monophyletic origin approximately contemporaneous with the origin of the Order. Had there been separate origins of the character within the several families or tribes it might be expected that several taxa within the Order would have retained the true single pollen condition, and this does not appear to be the case.

Any assumption of multiple or polyphyletic origins of monad type pollen in the Styphelioideae is not permissible. The type is not found elsewhere among the Angiosperms. Only in the Cyperaceae is there any parallel behaviour (Juel, 1900; Stout, 1912; Piech, 1928; Tanaka, 1940, 1941), and the details of pollen development in the two families are quite different. Within the Styphelioideae—and also within the Cyperaceae—the monad form must have had a single origin from a prior tetrad form, and this origin must have been contemporary with the origin of the tribe itself. The descriptions which have been presented earlier show that the monad pattern of development must be determined by a quantitative cytoplasmic system which must itself be under ultimate nuclear control. The whole system must be complex, and it would seem more likely that it could break down on several occasions than that it should have had several independent origins.

Since both full tetrad and monad pollen types occur within the tribe, and since the latter must be derived from an earlier tetrad condition, it could be supposed that the tetrad type, where it occurs within the tribe, is actually primitive. Alternatively, the hypothesis may be suggested that the tetrad type within the tribe results from the loss of the conditions necessary for monad development. The first view cannot be maintained. Table 8 summarizes the three-way relationship between genera, basic chromosome number and pollen types. The genera were established by Robert Brown, and although later botanists have not always recognized them as having generic status (Mueller, 1868; Drude, 1898; Maiden, 1916) they have never denied their reality as natural groups and have merely reduced them to sectional rank within a single genus *Styphelia*.

It has been inferred (Smith-White, 1955a) that the basic numbers in the Styphelioideae are  $x = 4$  and  $x = 6$ , and that the numbers 7, 9 and 10 are derived by polyploidy and secondary change. This view is necessary in *Astroloma* particularly, where the basic haploid number is 4 in fourteen species all with monad pollen, and where two species have  $n = 7$  and segregating tetrad pollen.

A consideration of the information in Table 8 shows that if the full tetrad T-type pollen is truly primitive within the tribe it is necessary to assume that the S-type has originated on several occasions or that the recognized genera have no phyletic meaning. It would also mean that the haploid numbers 7, 9 and 10 must be more primitive and that the numbers 4 and 6 must have originated several times, and always in association with an origin of the monad pollen type. Such a view is untenable.

Accepting the view that the origin of the monad type was contemporaneous with the origin of the tribe, and that the primitive basic number was  $x = 6$ , the S-type pattern was not upset by the origin of the 4-chromosome genom. The full tetrad T-type pollen in species of *Acrotriche*, *Lissanthe* and *Leucopogon* must then represent separate and independent breakdowns of the monad pattern, and it is significant that



such breakdowns are always associated with the origin of secondary basic numbers. Also, the S'-type of development, the partial breakdown in *L. virgatus*, and the segregating tetrads in twelve species belonging to four genera must represent stages in a transition back from monad to full tetrad pollen. This reversion does not do violence to the general concept of the irreversibility of evolution.

It is known that tetrad segregation in *Astroloma pinifolium* and in *Astroloma conostephioides* is associated with abnormal chromosomal conditions and behaviour during meiosis, and these abnormalities are in part responsible for the observed

TABLE 8.  
Genera, Pollen Types and Chromosome Numbers in the Styphelioideae.

CHROMOSOME NO. (n)	4	8	16	6	12	24	10	11	7	14	9	Un- known
GENERA												
Styphelia	M <sub>7</sub> <sup>S</sup>											
Astroloma	M <sub>14</sub> <sup>S</sup>	M <sub>1</sub> <sup>S</sup>	M <sub>1</sub> <sup>S</sup>						V <sub>2</sub>			
Conostephium		M <sub>2</sub> <sup>S</sup>										
Melichrus		M <sub>2</sub> <sup>S</sup>										
Leucopogon ♂ Pleuranthus	M <sub>7</sub> <sup>S</sup>	M <sub>1</sub> <sup>S</sup>		M <sub>5</sub>			M <sub>1</sub> <sup>I</sup>					
Leucopogon ♂ Perojoo				M <sub>4</sub>	M <sub>2</sub>	M <sub>1</sub>	MT <sub>1</sub>	M <sub>9</sub>				
Monotoca					M <sub>2</sub> <sup>D</sup>							
Cyathodes					M <sub>2</sub>		* <sub>2</sub> <sup>D</sup>				T <sub>1</sub>	
Leucopogon ♂ Heteranthesis										T <sub>1</sub> <sup>D</sup>	V <sub>1</sub> <sup>D</sup>	
Lissanthe									T <sub>2</sub>	T <sub>1</sub> <sup>D</sup>	V <sub>1</sub>	
Pentachondra										T <sub>1</sub>		
Brachyloma									V <sub>1</sub>		MT <sub>2</sub>	V <sub>2</sub>
Acrotriche											T <sub>3</sub> V <sub>1</sub>	V <sub>3</sub>
Trachocarpa							T <sub>1</sub>					
TOTALS NUMBERS OF SPP.	M <sub>28</sub> <sup>S</sup>	M <sub>6</sub> <sup>S</sup>	M <sub>1</sub> <sup>S</sup>	M <sub>9</sub>	M <sub>6</sub>	M <sub>1</sub>	MT <sub>2</sub>	M <sub>9</sub> <sup>I</sup>	TV <sub>2</sub>	TV <sub>3</sub>	MT <sub>25</sub>	V <sub>7</sub>

## LEGEND

M MONAD TYPE

V VARIABLE POLLEN TETRADS

M<sup>S</sup> MONAD TYPE Microspores initially unequal

T REGULAR FULL TETRADS

M<sup>I</sup> MONAD TYPE Microspores initially equal

D SPECIES WITH DIOECISM or PARTIAL DIOECISM.

SUBSCRIPTS— Nos. of Species

\* POLLEN TYPE UNKNOWN

segregations. In *Lissanthe strigosa* only a single plant amongst more than fifty is known to show segregation and in this species it is necessary to assume that the segregation is derived directly from the full tetrad condition. If, however, the monad type is the primitive one within the tribe, unusual cytoplasmic conditions must be associated with segregation, and in some of the species, particularly in *Astroloma* and *Brachyloma*, segregation must be derived directly from the monad type. It is probable that the behaviour, even when not directly derived from the monad type, is dependent upon a preadapting evolutionary history involving monad pollen, cytoplasmic differentiation or polarity, and possibly the cytoplasmic control of chromosome segregation. This view is further supported by the high frequency of the occurrence of tetrad segregation within the tribe compared with its low frequency in other families with

tetrad pollen, and by the quantitative intergradation which connects the extreme monad and the full tetrad types.

## 2. A Deductive Hypothesis.

The treatment of the problem of pollen development in the *Styphelieae* so far has been descriptive and inductive. The main conclusions which have been reached may be enumerated.

Firstly, all the patterns of pollen development in the tribe are related in causation. They constitute parts of one large problem. One general hypothesis must be capable of explaining and relating all the modifications of this causal system, and an attempt to formulate such an hypothesis is worth while.

Secondly, the monad pattern of pollen development has had a single origin, roughly contemporaneous with the origin of the tribe itself, and may be regarded as the primitive condition within the tribe. Although the monad type must have originated from an earlier tetrad condition, full tetrad pollen, where it occurs within the tribe, is derived by the breakdown of the monad type. Tetrad segregation must also be related to the monad type.

Thirdly, from a consideration of the implications of the *Styphelia* type of monad development, it is inferred that the control of the monad system is primarily cytoplasmic and there must be a cytoplasmic polarity within the mother cell. Cytoplasmic polarity must mean that certain vital constituents attain a gradient distribution within the general "body" cytoplasm of the cell. Chromosomal homozygosity may be assumed as an initial condition, and differential nuclear migration is determined by position.

Fourthly, the permanent triploid *L. juniperinus* demonstrates that unbalanced univalent chromosomes are sensitive to this gradient and control of their segregation follows as an immediate consequence. Any control of the segregation of homozygous paired chromosomes is impossible and meaningless, but such control may extend to structurally heterozygous bivalents provided this heterozygosity involves sensitive chromosome segments. Support for this suggestion comes from evidence discussed earlier (page 13), that preferential segregation is often a function of heterochromatin.

Fifthly, the triploid species also shows that a similar control operates in the embryo sac mother cells, but under the influence of a different tissue environment. The conditions necessary for complementary gametic selection are present and confer preadaptation to permanent hybridity.

Finally, cytoplasmic differentiation must be under genotypic control. It is quantitative and complex. Change in genetic balance consequent upon change in the basic genom predisposes to the loss of polarity in the mother cell.

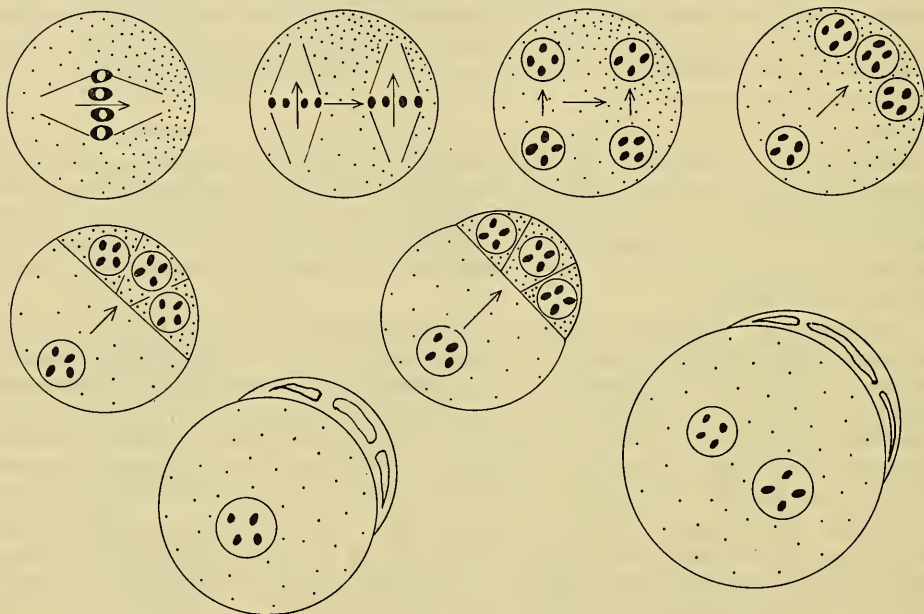
### *The Initial Operation of the System.*

These conclusions may be taken to constitute a fundamental system and an attempt will be made to deduce its evolutionary consequences. Text-figure 8 illustrates the immediate operation of the system in pollen development in a homozygous diploid with four pairs of chromosomes. Polarity causes a gradient distribution of certain cytoplasmic constituents (stippling) and also of non-chromosomal nuclear materials. Nuclear migration is one result. Microspore survival is determined by the positions of the nuclei in the mother cell; that microspore survives which receives an adequate supply of both the polarized materials and of the "body" cytoplasm, and this would obviously be the isolated nucleus at the "negative" end of the polarity, since, getting the major share of the body cytoplasm, it would also get sufficient of the polarized constituents.

The operation of the same system in the ovule is illustrated in Text-figure 9. Intracellular conditions in the P.M.C. and in the E.S.M.C. are the same, but the

closely enveloping ovule tissue maintains a linear arrangement of the second division spindles and of the megaspores, and precludes nuclear migration. An ovule tissue gradient, not present in the anther, permits the development only of the micropylar megaspore.

Conditions of megaspore competition are thus different from those of microspore competition. The intracellular E.S.M.C. polarity may favour either the micropylar or the chalazal ends. In the former case (Text-fig. 9, left) the micropylar megaspore receives a major share of the polarized cytoplasmic components and an equal quarter-share of the body cytoplasm. A functional embryo sac is produced. Where, however, the intracellular polarity favours the chalazal end and operates against the tissue gradient, the micropylar megaspore gets insufficient polarized constituents and aborts; the micropylar megaspore commences to develop, but fails at an early age, and no functional embryo sac is produced (Text-fig. 9, right). The direction of the intracellular



Text-fig. 8.—The control of pollen developing in a diploid species. A cytoplasmic polarity (stippling) determines nuclear migration and survival. Cf. text.

mother cell gradient is apparently independent of the ovule tissue gradient, since species with monad pollen show a maximum of 50% seed fertility.

The hypothesis set up is admittedly superficial, since the nature of the gradient cannot be suggested. It is, basically, an hypothesis of the interaction of two different kinds of competition, balanced against each other differently in the pollen and embryo sac developmental sequences. Functional pollen grains are developed only from the "negative" end of the cytoplasmic axis, and functional embryo sacs come only from the "positive" end of the same axis. If unbalanced chromosomes are sensitive to cell polarity there is preadaptation to the maintenance of permanent hybridity.

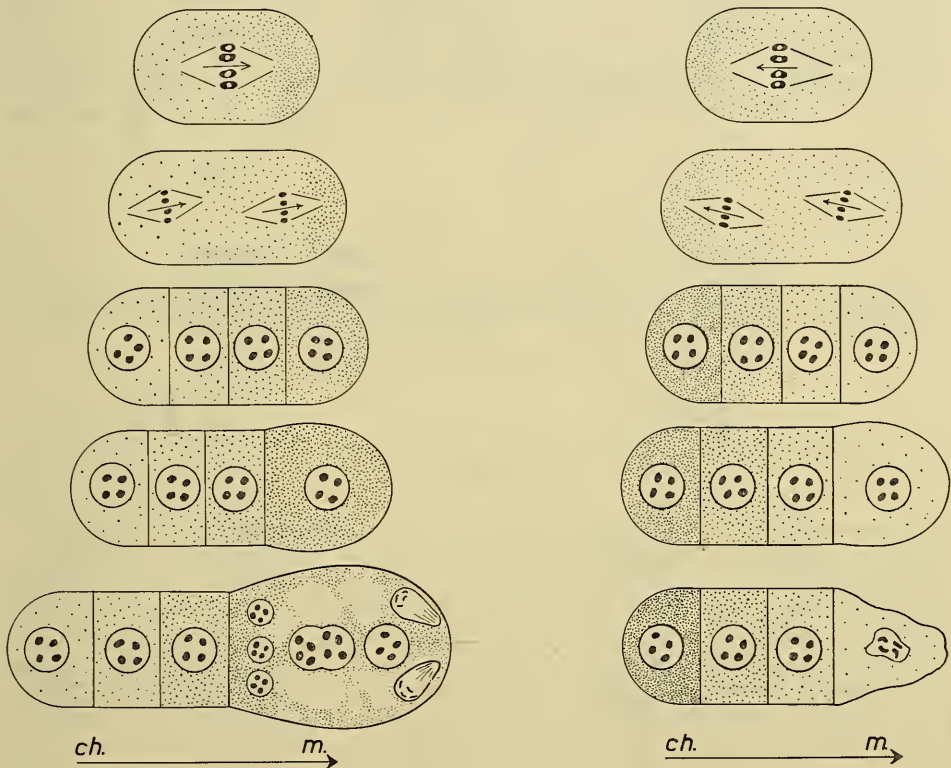
#### *The Operation of the System in the Triploid.*

The operation of this system in the triploid *Leucopogon* is illustrated in Text-figures 10 and 11, and these figures need very little further explanation. When the triploid was first formed, presumably following hybridization, the exclusion of univalents from the effective pollen, and their inclusion in the effective embryo sacs would be an immediate consequence of the prior conditions of pollen and embryo sac development.



This initial control probably is not the final one. The univalents would be subject to a predominantly maternal inheritance and, since lethal mutations are relatively frequent, they could in the course of time accumulate pollen lethals preventing the survival of the exceptional grains which might contain them. Selection would also favour the accumulation in them of genes favourable to embryo sac development, and there would then be no retreat from permanent hybridity.

It is possible that a similar system is responsible for the maintenance of univalents in the *Rosa canina* complex. In these roses there is no polarity in pollen development, and univalents are eliminated in the pollen line only by the intolerance of genetic unbalance. In the embryo sacs, however, conditions must be very similar to those in



Text-fig. 9.—The control of embryo sac development in a diploid species. There is interaction between an ovule tissue gradient favouring the micropylar megaspore (bottom arrows) and a cytoplasmic polarity (stippling). The latter may favour either end of the megaspore-tetrad. Cf. text.

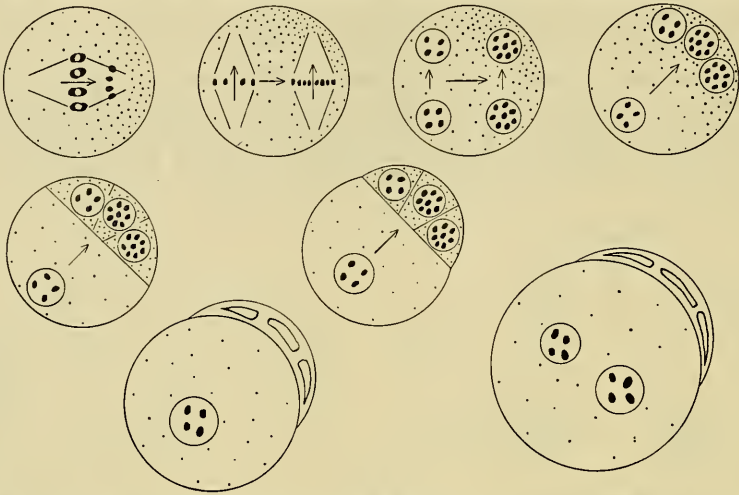
*L. juniperinus* and an initial tendency for the univalents to be inherited in the maternal line would then be strengthened by selection both of genes necessary for embryo sac development and of more reliability in polarized segregation, as suggested by Darlington (1939).

#### *The Origin of Permanent Hybridity in Diploid Species.*

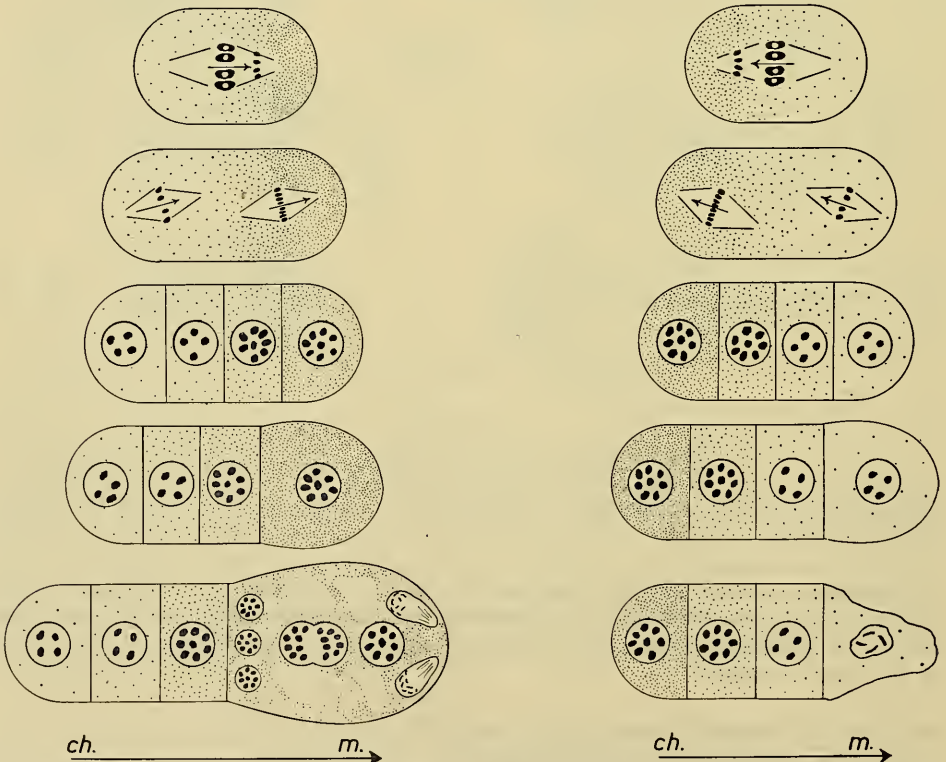
If, as has been inferred, cytoplasmic control of segregation can extend to structurally heterozygous bivalents, it would be possible for permanent structural hybridity to be established in diploid species possessing monad pollen. It will be

assumed that structural hybridity may arise adventitiously in any species, but that in sexual species with a normal genetic system it will usually be eliminated.

One necessary condition, for permanence, is some degree of chiasma localization, but this is not a serious restriction, since chiasmata are rarely or never strictly

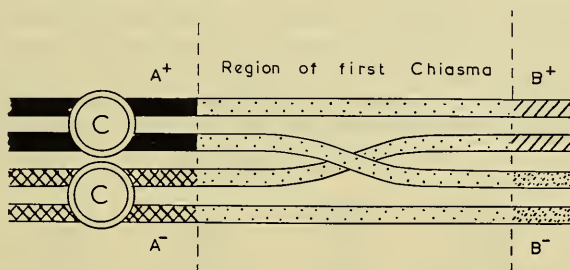


Text-fig. 10.—The control of pollen development in the triploid *Leucopogon juniperinus*. Univalents are "gradient-positive" and are excluded from the functional pollen. Cf. text.



Text-fig. 11.—The control of embryo-sac development in the triploid *Leucopogon juniperinus*. The univalents, being "gradient-positive", are included in the functional embryo sacs. The univalents of the triploid are strictly maternal in inheritance. Cf. text.

terminal or strictly adjacent to the centromeres. Text-figure 12 illustrates a bivalent in which there is one chiasma, with the proximal and distal regions excluded from the chiasma region. The bivalent can be extended to a second chiasma region without affecting the subsequent argument provided the first and second chiasma regions do not overlap, i.e. provided there is a degree of chiasma localization. An adventitious structural change, adjacent to the centromere, would cause an orientation of the bivalent at first metaphase, with the more sensitive segment directed towards the positive end of the cell polarity. As with the univalents in the triploid, this controlled segregation would favour the exclusion of the positive reacting chromatin from the pollen and its inclusion in the embryo sacs, and the negative responding chromatin would be favoured in the pollen. Similarly, an adventitious structural hybridity distal to the chiasma region, and perhaps with neocentric activity or acting indirectly through the centromere (Darlington, 1956), could cause a non-random orientation of the chromatids at the second division. It is not necessary that this control should be near-perfect in the beginning, but only that it should be substantial, since selection would then favour its greater stabilization. Adventitious structural hybridity would be maintained by the complementary gametic selection imposed by the cytoplasmic system.



Text-fig. 12.—Diagram of a bivalent with a single chiasma and some localization. The proximal and distal segments lie outside the chiasma region. Cf. text.

With this cytoplasmic system operative, a reinforcement with a genetic mechanism would be almost inevitable. Bennett (1956) has demonstrated that even in species with more normal genetic systems there is an appreciable prospect of the establishment of balanced lethal mechanisms within non-crossover segments. In the system suggested here there would be no selection against pollen-lethal mutations in the maternally inherited gradient-positive segments nor against embryo sac lethals in the paternal gradient-negative segments. This would lead to the reinforcement of the cytoplasmic control of the monad pattern of development by a more rigid system involving chromosomal segregation and permanent balanced gametic lethals. Referring again to Text-figure 12, if A and a represent "positive" and "negative" proximal segments, and if B and b represent similar segments distal to the chiasma region, we have precisely the segregational scheme mentioned and rejected earlier (Text-figure 2b).

There is an important complication, however. In *Styphelia* there are four pairs of chromosomes, and each is equally liable to the consequences of cytoplasmic polarity. In each bivalent there is a single proximal position (i.e. on both sides of the centromeres) and two distal positions (one on each chromosome arm) which are protected from crossing over and which are therefore potential sites for structural hybridity. With maximum complexity the constitution of a diploid *Styphelia* or *Astroloma* could be:

S A T	U B V	W C X	Y D Z
— — —	— — —	— — —	— — —
s a t	u b v	w c x	y d z

where the dots represent centromeres and the symbols represent "positive" (capitals) and "negative" (lower case) segments. This segregational system could reinforce,



but not replace, cytoplasmic control, since it would break down if subjected to random segregation. Further, it could not operate on the bivalents in the *caninae* roses, since there is no polarity and therefore no possible control of bivalent segregation, in the pollen mother cells of *Rosa*.

#### *The Breakdown of the System.*

It has been found that loss of the monad pattern is associated with secondary polyploidy and change of genetic balance. Secondary polyploidy does two things. It brings a reduplication of a large part of the genom and it causes a loss of polarity in the pollen mother cells. In *Astroloma*, with  $x = 4$ , the two species with A-type pollen both have  $n = 7$ . Assuming the full structural hybridity illustrated above, their constitutions would become

S A T	U B V	W C X	Y D Z	U' B' V'	W' C' X'	Y' D' Z'
—	—	—	—	—	—	—
s a t	u b v	w c x	y d z	u' b' v'	w' c' x'	y' d' z'

It is not necessary to assume that genetic differentiation should occur in all seven proximal positions and all 14 distal positions. The loss of cell polarity would open the system to segregation. Assuming segmental heterozygosity in all positions except those in the unrepeat chromosome, and either duplicate or alternate interactions between the repeated parts of the genom (i.e. either auto- or allopolyploidy), pollen tetrad segregation would result, with frequencies of the five possible tetrad types:

#### Percentage frequencies.

Interaction	nullads	monads	dyads	triads	full tetrads
Duplicate	75.73	24.64	2.50	0.127	0.003
Alternate	97.10	2.83	0.022	0.0002	0.000007

Thus, following loss of polarity, the initial pollen fertility would be extremely low provided full complexity had been attained. However, selection should tend gradually to undo the system previously built up under the protection of polarity, and simpler patterns of segregations such as those actually observed would result. Ultimately, the whole segregating system might be eliminated, giving reversion to the full tetrad type. It would be expected that the interaction relationships of the reduplicated genoms would be different in the several independent origins of secondary ploidy within the tribe. Also, the selective reduction of the segregating system would vary in each case; it would be rapid in the absence of the accumulation of pollen and embryo sac lethals on the "negative" and "positive" segments respectively, but slow and perhaps almost impossible in the event of such accumulation. The different species would show differences in the ease and rate of reversion to the full tetrad condition.

#### *The Origin of Dioecism and Mixed Sexuality.*

The chromosomal system wherein certain segments are inherited solely through the embryo sacs and where alternative segments are solely paternal, has some similarity with ordinary sex mechanisms. A very simple system of mixed sexuality can be deduced on such a basis.

In the simplest case, structural hybridity A/a may be assumed in a single segment, where A is positive to cell polarity and a is negative. Segment A has accumulated deficiencies which act as gametic lethals in the pollen, and similarly a has accumulated embryo sac deficiency lethals. With secondary ploidy there is a reduplication of these segments, giving either the constitutions A/a A/a (autopolyploidy) or A/a A'/a' (allopolyploidy). This is the simplest form of the system already deduced. There is also a loss of polarity. The only new assumption necessary is that in individuals homozygous for A/A or A'/A' there is anther abortion, and that in individuals a/a or a'/a' there is pistil abortion. Three genotypes of embryo sacs, AA', Aa', and aa', and three of

pollen grains,  $Aa'$ ,  $aA'$ , and  $aa'$ , are possible, and in the sporophyte there are seven genotypes and four phenotypes:

$\frac{AA}{Aa'}$	$\frac{AA}{aa'}$	$\frac{AA'}{aa'}$	$\frac{Aa'}{aa'}$	$\frac{aA'}{aa'}$	$\frac{Aa'}{Aa'}$	$\frac{aA'}{aa'}$
Female		Her- maphrodite		Male	Neuter	

There is an interesting feature of this system. Starting with any frequencies of the seven possible genotypes, and in the absence of selection, the system will rapidly approach a stable equilibrium in which the proportions  $p$  and  $q$  of  $A$  and  $a$ , and  $p'$  and  $q'$  of  $A'$  and  $a'$ , are equal, and in which the four phenotypes have the proportions 0.24 females, 0.35 hermaphrodites, 0.24 males and 0.17 neuters, which may be further summed to 0.59 male-fertile and 0.41 male-sterile. These are close to the actual proportions observed in *Lissanthe montana* at Kosciusko. The occurrence of such a stable equilibrium, which of course might be modified by other factors of selection, would mean that the system could be readily established once the necessary prior conditions were provided. In this respect it resembles the relatively simple case where selection favours a heterozygote  $A/a$  over either homozygote  $A/A$  or  $a/a$ .

The system also resembles fairly closely the two-factor mechanisms which have been described in *Rubus idaeus* and in *Vitis*, referred to earlier in this address. However, it is capable of much greater complexity, where heterozygosity might be established in similar non-crossover segments in other chromosomes, and it is capable of giving a complex sex-strength system which seems to be necessary in *Lissanthe montana*. It is also capable of being modified in the direction of complete dioecism.

#### SUMMARY.

The most frequent, and perhaps the most characteristic pollen type in the Stypheleae is the monad. Monad pollen is derived from the tetrad form by the regular failure of three microspores in the young tetrad. In its most extreme form, monad development involves nuclear migration in the mother cell following the conclusion of meiosis.

Other pollen types found in the tribe include modified monads where nuclear migration is absent, variable pollen tetrads, and regular pollen tetrads.

An attempt has been made to show that all pollen types in the tribe must be related at the level of causation, and that the extreme monad type is the basic one within the tribe. Its establishment must have been roughly contemporaneous with the origin of the tribe. The other pollen types represent breakdown or loss of the monad pattern of development.

The monad pattern of development necessarily implies the development of cytoplasmic differentiation or polarity within the pollen mother cell. This polarity may possibly be related to that which is normal in angiosperm pollen grains.

Conditions in a permanent triploid *Leucopogon* demonstrate that this polarity is present in the mother cell during the first meiotic division, and also that unbalanced chromosomes (univalents) may be sensitive to it. The permanent triploid also demonstrates the existence of a mechanism of complementary gametic selection. This mechanism must be antecedent to the origin of triploidy and its presence can be inferred in diploids.

Using these inductive conclusions, an evolutionary hypothesis has been presented to explain and relate the different pollen types. According to this hypothesis, structural hybridity could be maintained in diploid species by a mechanism similar to that demonstrated in the triploid. Loss of polarity, accompanying secondary ploidy and change of genetic balance would then give rise to the variable tetrad pollen type.

The hypothesis permits the deduction of a system of mixed sexuality and unstable dioecy which is adequate to explain actual conditions in a number of species of the tribe.

The hypothesis is admittedly a speculative one. Its only merit may be that it permits a unification of several problems within the group. It is, however, capable of experimental testing, and I hope to be able to contribute in this way, in the future, either to its establishment or its downfall.

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## EXPLANATION OF PLATES I-II.

## Plate i.

Breakdown of monad development in *Leucopogon virgatus*, Plant R57/10.

## Plate ii.

Variable Tetrad Pollen.

1. *Astroloma pinifolium* × 150.
- 2, 3. *Astroloma conostephioides* × 150.
4. *Acrotriche fasciculiflora* × 300.
5. *Leucopogon melaleucoides* × 300.
6. *Brachyloma ericoides* × 300.